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THE MEGASPOROPHYLL OF SAXEGOTHAEA AND MICROCACHRYS

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(WITH PLATES XXII–XXV)

In the Coniferae great importance attaches to "inverse orientation" as a criterion for determining the character of the fertile scale in the megasporangiate cone. The followers of BRAUN see in the inversion of the ovuliferous scale bundles an indication of the "brachyblast" character of this structure. SACHS and EICHLER, the exponents of the "ligular" theory, look upon the inversion as a feature which characterizes the vascularization of a ligule or of an appendage of the simple sporophyll. CELAKOVSKY partly combines the two views and seems to homologize many of the diversified megasporangiate features of the gymnosperms. In the present study some neglected phases of the inversion of the sporangial supply bundles of both the staminate and ovulate cones are given prominence, and data are advanced to show the homology of the micro- and megasporophyll in Saxegothaea and Microcachrys.

The gross features of a fruiting branch of Saxegothaea are indicated in *fig. 1*. The megasporangiate cones are borne terminally on the branches (*figs. 1, 2*), the microsporangiate ordinarily in the axils of foliage leaves, but occasionally in a terminal position (*fig. 3*). The megasporangiate cone, at the stage indicated in *fig. 2*, has a short pedicel beset with the bracts which earlier inclosed it. Later the pedicel elongates and a lax arrangement of the bracts is evident (*fig. 4*). Still later the pedicel becomes much more elongated and relatively very slender. The bracts, sporophylls, and foliage leaves are spirally arranged, and gradations in form are evident, the bracts gradating

apically into sporophylls and basally into vegetative leaves (*fig. 4*). The microsporangiate cones may be either sessile or pedicellate. In either case there is a series of spirally arranged bracts closely investing the base of the cone, the lower part of the pedicel, when present, being naked (*figs. 2, 3*). In an otherwise abnormal cone I found the arrangement of bracts similar to that on the megasporangiate pedicel.

The gross features of Microcachrys are indicated in a figure of a previous article.[1] The cones are always terminal in this form and their sporophylls verticillate, in series of fours, the series alternating with one another and presenting from the exterior an appearance of a spiral arrangement (*figs. 5, 6*). The small concrescent foliage leaves are opposite, in alternating pairs.

The form and structure of the microsporophyll of Saxegothaea are indicated in *figs. 7–9*. Two are shown cut longitudinally (*fig. 7*), and between these is one cut through the sporangium, showing the stomium and the wall with its columnar, comparatively thick-walled, epidermal cells. The inner layers of the wall have collapsed, but there are indications of three or four of these.[2] In the axial sections the vascular bundle with its accompanying resin duct is seen, and the connection of these with those of the axis of the cone. The resin canal is expanded distally. This is apparent in the tangential section of the cone as well (*fig. 9*), where the central sporangia often show little trace of a canal, while in the lateral ones it is large. In *fig. 8* resin canals and vascular bundles are seen in transverse sections of the cone, at various distances from the axial supply. In some of the sporangia, to the right of the figure, the position of the stomia is indicated. The microspores contain three cells at the stage from which these figures were made. Megasporangiate cones of the same date have not differentiated the megaspore (*figs. 10, 11*), with the exception of some abnormal ovules, to be described later. A single bundle accompanied by a resin canal passes into each sporophyll, and this gives off the ovular supply just as the point of insertion of the integument is reached

[1] THOMSON, R. B., On the pollen of Microcachrys. BOT. GAZETTE **47**:26–29. *pls. 1, 2.* 1909. See *pl. 1.*

[2] Cf. NORÉN, C. O., Zur Kenntnis der Entwicklung von *Saxegothaea conspicua* Lindl. Svensk. Bot. Tidskr. **2**:101–122. *pls. 7–9.* 1908.

(*fig. 11*). *Fig. 12* is a transverse section of the sporophyll a little nearer the cone axis than the point indicated. It shows a single vascular bundle, and below it the accompanying resin duct. The wood (lighter in the figure) is roughly triangular in outline. Near its center are two very darkly nucleate cells. These mark the separation of the wood of the ovular supply from that of the main bundle. The bast above this is but slightly differentiated, and nearer to the axis of the cone dies out completely, as does the wood itself in its further course through the cortex. Under the base of the ovule the bast is quite apparent on the upper side of the supply bundle (*fig. 11*), which shows a tendency to bifurcate, the branches passing laterally into the base of the integument. Beyond the separation of the ovular supply, the main bundle gradually acquires a new set of centripetal wood elements, the more central ones of which are elongated, the lateral quite typical transfusion tissue.

In material of Saxegothaea some six months older than the former, the vascular system of the megasporophyll is further developed. It has been recently described by Miss STILES,[3] with whose account my own observations are practically in accord. The upper vascular system may be composed, in the region of the ovule, of as many as four strands. These, however, in all the material I examined, unite into two before passing upward into the base of the ovule. Miss STILES speaks of there being about three bundles in this region. The pair terminates at the level of the base of the nucellus in a considerable expansion of transfusion-like tissue. The main bundle of the sporophyll is replaced by a series somewhat similar in the same region in which the proliferation of the ovular set occurs. Above the ovule a single bundle is found, with centripetal, and on its flanks a considerable, development of xylem elements. The vascular supply for the scale cones from the axis is a single bundle with normally oriented wood and bast. In the cortex gradually new xylem elements are formed opposite the protoxylem. Farther out wood and bast coming off the sides of the main bundle supplement the original central elements, which up to this point appear not to have any bast of their own. In the further course the proliferation of the upper and

3 STILES, W., The anatomy of *Saxegothaea conspicua* Lindl. New Phytol. **7**: 209–222. 1908.

lower series occurs, as described above. All of the upper series of bundles pass into the base of the ovule, the only xylem elements found beyond the ovule in this region being a new development associated with the main bundle. A single resin duct accompanies the latter throughout its course. This does not branch and is the only representative of the tissue in the scale, no canals being formed in connection with the upper series of bundles.[4]

The vascular system of the axis of the megasporangiate cone consists of a ring of collateral bundles very similar in general to that of the microsporangiate cone (*fig. 8*). The wood of these is usually of the ordinary endarch type, but near the base of the cone there are often a few centripetal elements associated with the bundles at the sides of the gap left by the exit of the megasporophyll trace. In one instance these were almost in continuity with the wood elements of the ovular supply in the sporophyll. The latter, especially in its young condition, appears very much of the nature of centripetal xylem (*fig. 12*),[5] and the occurrence of the isolated elements in the axis affords confirmation of this idea. They are found also in the upper part of the pedicel, but in my material they are rare here.

The scales at the base of the micro- and megasporangiate cones and the foliage leaves both receive a single vascular bundle and a single resin duct from the axis, the supply coming off in a similar way to that of the sporophylls. There is always a gap in the cylindrical stele opposite the "trace" to each of these members (*fig. 8*, for microsporophylls, lower right hand side, etc.).

In several of the megasporangiate cones of Saxegothaea a few of the lower sporophylls bear ovules on their under surface (*fig. 10*). These have an integument, but not the epimatium or rudimentary second integument of the normal ovule. They stand out too from the sporophyll more freely, and are further developed than the upper ones in the same cone. A slight vascular supply goes off to some of these, the bast and wood showing a tendency to orient itself inversely to that of the main bundle of the scale. This supply passes around the resin duct.

4 Cf. STILES, *l. c.* 216.

5 Miss STILES has come to a similar conclusion from studying the more mature condition.

In Microcachrys a single vascular bundle supplies leaf and sporophyll. There are no resin canals in the stem nor in the axis of either cone. It is not until well out into the sporophyll that a canal is found beneath the vascular bundle (megasporophyll, *fig. 13*). The canal of the vegetative leaf, however, is continued downward into its swollen base, and the leaves being concrescent, there is the appearance of resin ducts in the stem. The vascular bundle in both leaf and sporophyll runs closer to the upper than the lower surface. A comparison of longitudinal sections of the megasporophyll and vegetative leaf shows a great resemblance between the distal part of the former and the whole of the latter. There is a distinct palisade on the lower surface of each, a sclerotic hypoderma, and an epidermis with a very thick cuticle and no stomata, the latter being restricted to the upper surface, and in the case of the sporophyll, so far as I have observed, to the region beyond the ovule. There is no hypoderma on the upper surface, and the same kind of degenerate-looking palisade tissue occurs in both sporophyll and leaf. Proximally the sporophyll is contracted into a slender stalk, while the leaf is concrescent with the stem. The vascular supply of the ovule originates in much the same way as in Saxegothaea with at first no apparent bast[6] (*fig. 14;* above the resin canal come bast and wood of the main bundle and above the center of the latter the few wood elements of the ovular supply). On separation the ovular supply bundle passes obliquely upward, as in Saxegothaea (cf. *figs. 11* and *13*), with bast clearly evident on its upper side. In Microcachrys, the single supply bundle bifurcates near the base of the ovule, the divisions passing well into the basal part of the integument.

In most of my material the megaspore of the ovules has only a few nuclei in a parietal stratum of protoplasm (*fig. 13*). In one case, however, a prothallial tissue (*fig. 15*) is present with the archegonial initials discernible. These are located at considerable distances from one another. Around the prothallium the megaspore-coat is of the usual double type.[7] It is slightly thicker than in *Pinus resinosa* at a similar stage. Like the pine coat at this young stage, it is of uniform dis-

[6] Cf. WORSDELL, Observations on the vascular system of the female flowers of the Coniferae. Annals of Botany **13**:538. 1899.

[7] THOMSON, R. B., The megaspore-membrane of the gymnosperms. Univ. of Toronto Studies, Biol. Ser. **4**:1–64. *pls. 1–5*. 1905.

tribution about the prothallium. It would be interesting to know the condition in the mature seed, especially in view of the fact that in certain species of Dacrydium the megaspore coat is very thick at a later stage.

The integuments of the ovule are seen in their usual form in *fig. 13*. Sometimes the inner one is quite open above the ovule (*fig. 15*), even at an advanced stage. In the case figured it is developed laterally into two large masses, appearing as additional ovules in gross material. In other cases it may be uniformly quite thick (*fig. 16*). The two outer cell layers of the integument are tangentially elongated in the older condition, while the third consists of columnar cells radially placed, many of which in the basal region become lignified and have numerous large pits. They are in connection with the supply bundles of the ovule through transfusion-like extensions of the latter.

Fig. 17 is of a longitudinal section of the bract and scale from the megasporangiate cone of *Tsuga canadensis*. The section passes to one side of the axis in the region of the ovule. To the lower right-hand side of the figure the bract is seen fused proximally with the tissues at the base of the scale. The latter shows one large vascular bundle, cut nearly transversely. This has the wood below and the bast above, an arrangement the inverse of that in the bract. From this bundle is given off the ovular supply, which passes obliquely upward into the base of the sporangium. The character of the supply bundle was studied in series of transverse sections. It comes off from one of the lateral bundles of the scale (*fig. 15;* second bundle from the right and left of the figure). Its wood and bast lie at first in a plane at right angles to that of the scale bundles (*fig. 19;* the wood is the small-celled tissue in the center of the figure and the bast is to the left). Shortly the bundle turns completely round, so that its wood lies above and its bast below, the inverse arrangement to that of the scale bundles, which, in turn, is inverse to that of the bract. Near the ovule the bundle becomes concentric and finally bifurcates, the branches entering the base of the integument.

In the microsporophyll of the cycads THIBOUT[8] has called attention to the inverse orientation of the sporangial supply. I have

[8] THIBOUT, E., Recherches sur l'appareil mâle des gymnospermes. pp. 265. *pls. 16*. Lille. 1896.

figured that of *Ceratozamia mexicana* (*fig. 21*). In *fig. 21* the upper bundle is one of the normally oriented main bundles—bast below and wood above, not shown in the section—while cutting obliquely across the field is a sporangial supply bundle with bast above and wood below. A short ventral extension to the sporangia turns at right angles at first and then becomes almost inverse. *Figs. 22* and *23* are transverse sections of a sporophyll. In the upper part of the figures are the main bundles, with wood above and bast below. Farther down and toward the ends of the normal series are some inversely oriented bundles. One, to the left, magnified in *fig. 23*, has just divided into a similarly oriented bundle, and from this there is a strand to the sporangium. The latter has a more or less concentric character. In some cases I have found a second inversion on the way to the sporangia.

The inversion of microsporangial supply bundles is of wide occurrence in the cycads, but is not confined to the group, being also found in certain conifers. This feature has not been considered by the exponents of the theories advanced to explain the inversion in the fertile scale of the latter. That it is of first importance seems self-evident. Again, the double inversion in the fertile scale has not been explained, and a glance at the figures shows how important the second inversion is, both of itself and in comparison with the single inversion in either Saxegothaea or Microcachrys (cf. *figs. 11*, *13*, and *17*). This and the occurrence of ovules on the lower surface of the scale in Saxegothaea, and their vascularization from the main supply, would seem to be good reason to consider that inverse orientation cannot in all cases be relied upon as evidence of the brachyblast character of the fertile scale. In the last case, we should have the anomaly of the leaf in the axil of the shoot.

Again, in Dacrydium two bundles with orientation inverse to that of the scale supply the ovule. These attach directly to the axis, the three passing close together near the upper surface of the scale. There is, then, in Dacrydium, a form admittedly of a higher type than either Saxegothaea or Microcachrys because of the reduction of the cone, etc., a greater amount of inversion than in the more primitive forms—a puzzling feature certainly in view of the brachyblast theory, but possibly finding its explanation in the greater

importance of the ovule in this specialized fructification and in the greater advantage which a direct connection with the axial supply affords. This has its beginning in Saxegothaea and Microcachrys, where the two bundles of the ovular supply have asserted their individuality only part way to the axis, the ovular supply in these forms having possibly originated, as has been suggested, as a revival of the centripetal development of the main bundle.

In view of the facts presented, the writer is led to look upon the fertile scale as a simple structure, the homologue of the microsporophyll in both Saxegothaea and Microcachrys, and, *ipso facto*, in the whole of the Taxaceae, if these form a natural alliance, as is commonly held. With this group is to be associated the Araucarieae, whose microsporophylls show an inversion of the sporangial supply bundles of like character to that of the megasporophyll,[9] and which, on other grounds, have been considered[10] as having a simple megasporophyll. This group also has recently been shown to have many features in common with the lower podocarps. Perhaps the most important of these are the method of pollination and the absence of wings on the grains themselves in Saxegothaea and the Araucarieae, the extensive microgametophyte of the two groups, the single inverted ovule, the wide micropyle and projecting nucellus, the nucellus free from the integument to its base—features which bring these forms into intimate association.

The simple-scale groups, the Taxaceae and Araucarieae, have then their micro- and megasporangia on opposite sides of the sporophyll; and since the homosporous forms, from which the conifers were derived, the pteropsid series, as is shown on morphological and anatomical grounds, have the sporangia usually on the lower surface, the ovule has probably been transferred to the upper surface in the course of phylogeny. The ovules on the lower surface of some scales of Saxegothaea and their lateral position in Cycas are significant in this connection, and, as well, the lateral development of the sporangium in Schizaeaceae.

[9] THOMSON, R. B., The origin of the gymnosperms at the Linnean Society. New Phytol. **5**:145. 1906.

[10] SEWARD, A. C., AND FORD, S. O., The Araucarieae, recent and extinct. Phil. Trans. Roy. Soc. London B. **198**:305–411. *pls. 23, 24.* 1906.

COULTER and CHAMBERLAIN, after reviewing the different theories of the fertile scale in their textbook on the gymnosperms, make the following statement (p. 77): "Upon sifting the testimony certain things seem to be fairly clear, and one is that the scale and its ovules in Abieteae represent a highly modified axillary shoot, corresponding to the characteristic spur shoot of the group." From anatomical and teratological evidence this conclusion seems inevitable, and since the Abietēae, Taxodieae, and Cupresseae present such a series of natural affinities the statement must apply to all. If, then, the fertile scale in this group is of the brachyblast type, representing the adaxially fused first and only two leaves of an abortive axis, the first inversion is explained, and the ovules in this group are borne on the morphologically under surface. The second inversion is analagous to the single one in Saxegothaea and of the nature of a sporangial supply. There are, then, two great groups of the conifers from the standpoint of this study, the simple- and the complex-scale series. Both forms have the ovules on the physiologically upper surface, a position rendered almost imperative by the necessities of the seed habit. This position, however, has been attained in two very different, but possibly equally difficult, ways in the aplo- and diplosporophyllous forms of the Coniferae.

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EXPLANATION OF PLATES XXII–XXV

PLATE XXII

FIG. 1.—Fruiting twig of *Saxegothaea conspicua*. Slightly enlarged.

FIGS. 2, 3.—Micro- and megasporangiate cones. ×4.

PLATE XXIII

FIG. 4.—Megasporangiate cone with the axis more elongated than in *fig. 2*.

FIG. 5.—*Microcachrys tetragona;* micro- and megasporangiate cones, the former in mature condition. ×4.

FIG. 6.—Older megasporangiate cone.

FIGS. 7–9.—Respectively radial, transverse, and tangential sections of the microsporangiate cone of Saxegothaea.

FIGS. 10, 11.—Megasporophylls of Saxegothaea, with ovules attached; *fig. 11* showing ovular supply coming off the main vascular bundle.

PLATE XXIV

FIG. 12.—Transverse section of the megasporophyll of Saxegothaea, young condition, just proximal to the insertion of the ovule.

FIG. 13.—Longitudinal section of the distal part of the megasporophyll of Microcachrys.

FIG. 14.—Transverse section of same, showing ovular and main supply bundles in transverse section.

FIG. 15.—Megasporangium of Microcachrys in transverse section.

FIG. 16.—Sporophyll and megasporangium with abnormally thick integument, in transverse section.

FIG. 17.—*Tsuga canadensis;* bract, scale, and ovule in longitudinal section.

PLATE XXV

FIG. 18.—*Tsuga canadensis;* transverse section of bract, scale, and ovules at the point of origin of the ovular bundles.

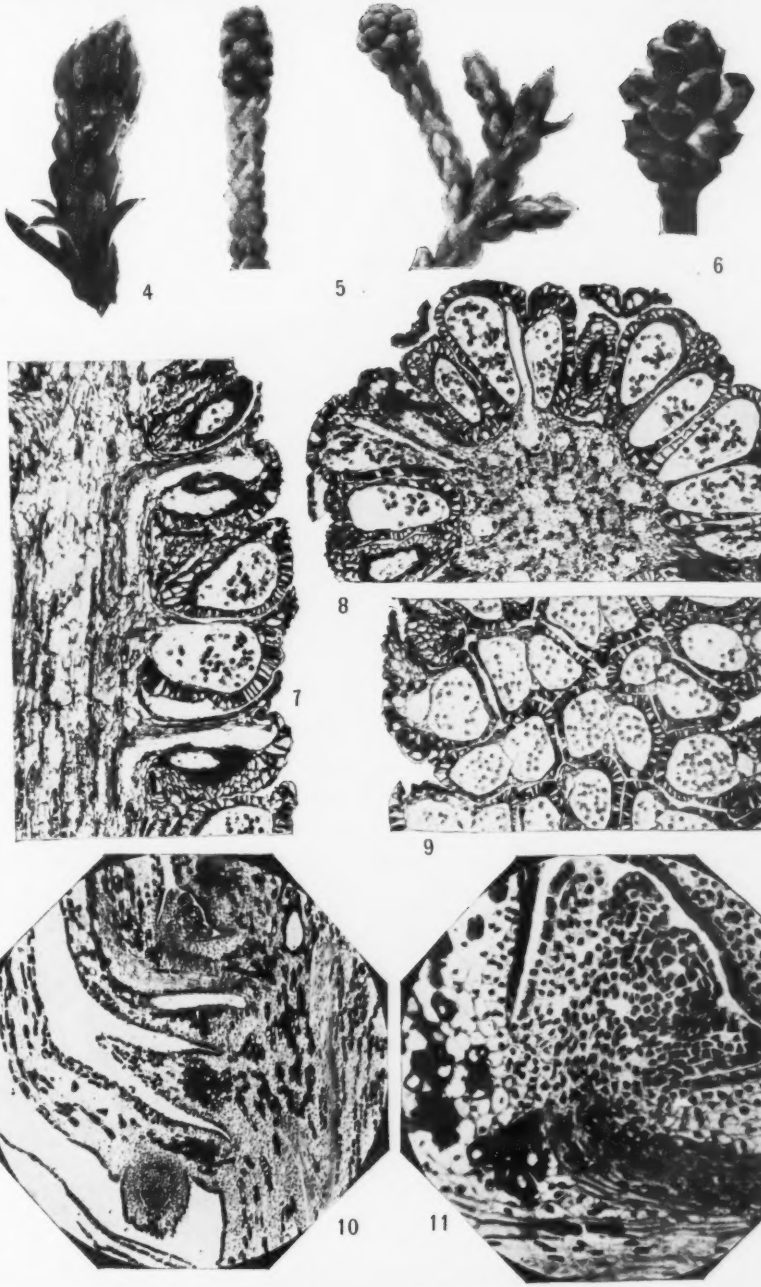
FIG. 19.—The same; a magnification of the ovular supply of the right ovule in *fig. 18.*

FIG. 20.—The same; supply bundle farther up.

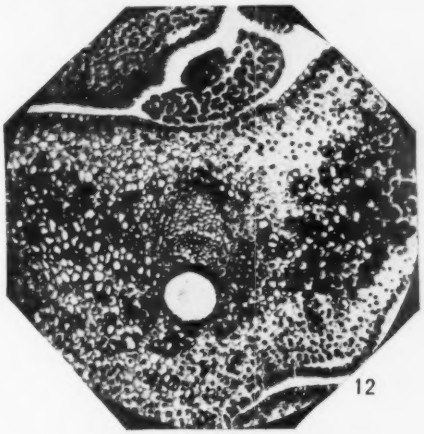
FIGS. 21, 22.—Respectively, longitudinal and transverse sections of microsporophyll of *Ceratozamia mexicana.*

FIG. 23.—A magnification of a part of *fig. 22.*

THOMSON on MEGASPOROPHYLL



THOMSON on MEGASPOROPHYLL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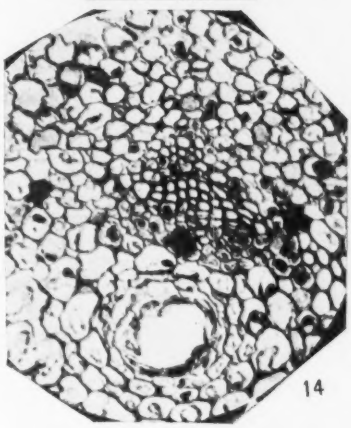
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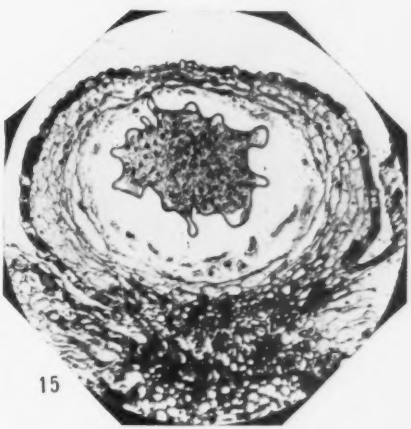
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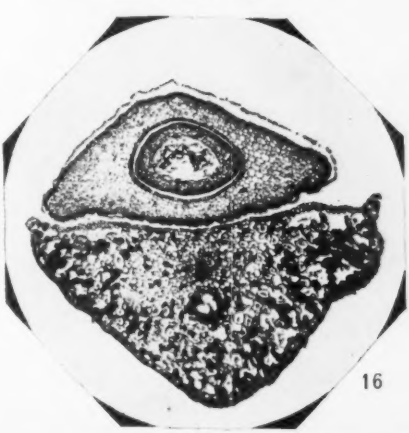
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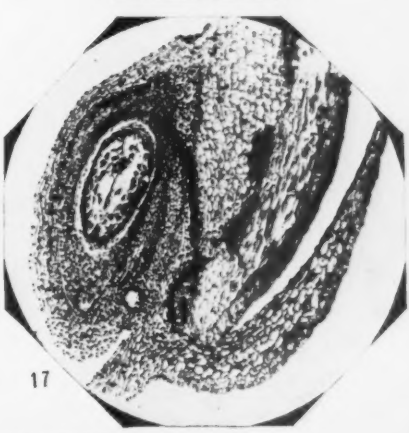
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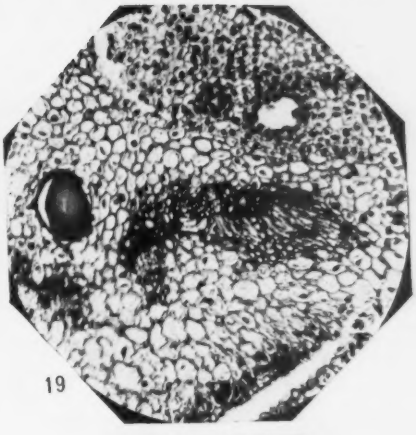
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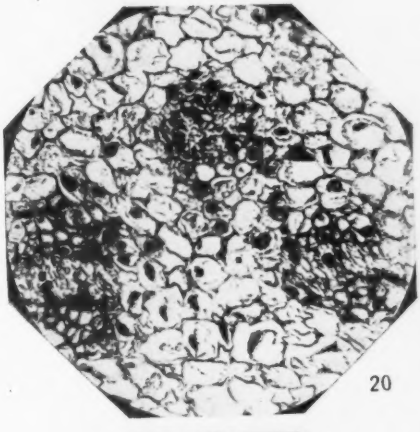
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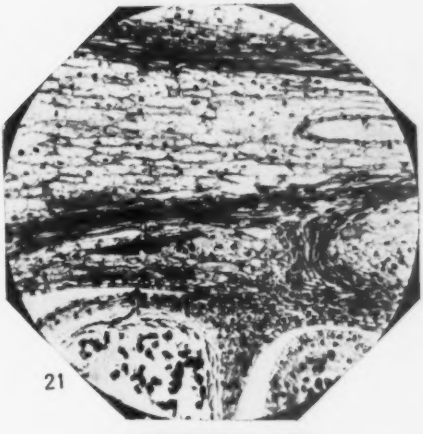
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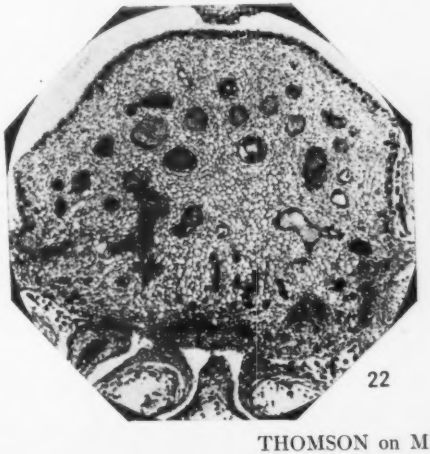
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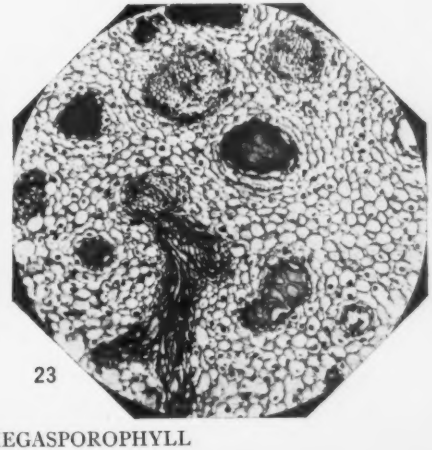
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THOMSON on MEGASPOROPHYLL

STUDIES ON THE OXIDIZING POWER OF ROOTS[1]

OSWALD SCHREINER AND HOWARD S. REED

The present paper embodies a series of studies upon the oxidizing powers of plant roots grown in aqueous extracts of soils and in solutions of various compounds. The results, it is believed, throw some light on the action of plants upon the soil and indicate how soil conditions affect certain functions of the plant.

The experiments which are presented show that plant roots are able to carry on active extra-cellular oxidation, chiefly by means of the enzymes which they secrete. From the standpoint of root excretions the study is of interest because it has often been supposed that the roots of growing plants excrete organic and inorganic acids which aid in the solution of soil minerals. The idea undoubtedly owes its prevalence largely to the experiments of LIEBIG[2] and of SACHS[3] which demonstrated the corrosion of polished marble plates by growing plants. The more recent investigations upon the subject made by CZAPEK,[4] KOSSOWITCH,[5] and KUNZE[6] have shown, however, that very little acid is excreted by the roots of the higher plants, and that the results of the earlier workers were mainly due to the action of carbon dioxid.

This oxidizing power of plants gives them an important action upon the soil. Whether they have the power to oxidize the inorganic constituents of the soil remains to be determined; but it has been shown that they are able to oxidize organic substances, such as the chromogens employed in these experiments, at a fairly rapid rate. If these substances are oxidized, it is only logical to conclude that the organic substances occurring in the soils are also oxidized by the action of plant roots.

This oxidation of organic compounds is of additional interest in the light of recent investigations, which show that the cause of unpro-

[1] Published by permission of the Secretary of Agriculture.

[2] Annalen Chem. Pharm. **105**:139. 1858.

[3] Bot. Zeit. **18**:117. 186c.

[4] Jahrb. Wiss. Bot. **29**:321. 1896.

[5] Ann. Sci. Agron. II. **8**:220. 1903.

[6] Jahrb. Wiss. Bot. **42**:357. 1906.

ductivity in certain soils is due to the presence of toxic organic compounds.[7] The beneficial effect of oxidation in such soils may be inferred from the results of thorough tillage, involving sub-drainage and cultivation, since these operations promote aeration of the soil with subsequent increase in growth of roots and microorganisms. Under such conditions experience has shown that the organic substances in the soil are most completely converted into substances commonly known as humus. It is certain that the oxidizing activities of the soil and plant play a significant part in this important process.

PREVIOUS WORK

The existence of an oxidation process in soils has long been known. Without apparently understanding the precise cause of this phenomenon, LIEBIG pointed out its importance for productive soils, and, according to the same author, the phenomenon had been earlier investigated by INGENHOUSS and DE SAUSSURE. Among the more modern investigations on the processes of oxidation in soils the works of DEHERAIN and DEMOUSSY,[8] WOLLNY,[9] RUSSELL,[10] and DARBISHIRE and RUSSELL[11] should be mentioned. These investigations are all along the line of bacterial activities in the soils. Recent studies by KÖNIG,[12] however, furnish evidence of a catalytic power of soils due to the presence of an enzyme.

The literature dealing with the oxidizing power of plant juices is already voluminous. Within recent years, our knowledge of processes going on within the plant has been greatly extended by the studies which have been made upon oxidizing enzymes. Since several comprehensive and instructive summaries of work on this subject have appeared, among which may be mentioned those of BACH and of CZAPEK's *Biochemie*, it is unnecessary in the present paper to review what has been done in this rapidly developing field.

[7] Jour. Amer. Chem. Soc. **30**:1295, 1599. 1908. Bur. Soils, U. S. Dept. Agric. Bulls. 36, 40, 53.

[8] Annales Agron. **22**:305. 1896.

[9] Die Zersetzung der organischen Stoffe und die Humusbildung. Heidelberg. 1897.

[10] Jour. Agr. Sci. **1**:261. 1905.

[11] Jour. Agr. Sci. **2**:305. 1907.

[12] Landw. Versuchsst. **63**:471. 1906; **66**:401. 1907.

The study of the oxidizing enzymes which are given off by the roots of plants, i. e., extra-cellular oxidation, has received less attention, and it is to this particular field that the present study belongs.

MOLISCH[13] appears to have been the first to demonstrate the oxidizing power of root secretions and to show their enzymotic nature. He found that the root secretion was capable of oxidizing various organic substances, such as guaiacol, pyrogallol, and gallic acid. His work showed that there was considerable active secretion on the surface of growing roots, and that this secretion had definite powers to effect changes in organic substances.

CZAPEK,[14] in making a general study of root secretions, followed some of the investigations made previously by MOLISCH. From experiments upon the action of seedling roots upon starch paste and sugar solutions, he regarded it probable that the growing roots produce only diastase or inverting ferments, although exact proof could not be offered. He believed, however, that the experiments of MOLISCH failed to prove the production of oxidizing enzymes by roots.

The ideas of the oxidizing powers of roots set forth by MOLISCH are well corroborated by the investigations of RACIBORSKI[15] upon the oxidizing powers of plant tissues.

In his work reagents were used which were so nearly non-toxic that they could be added to solutions in which plants were grown. In some experiments the reagents were added to water cultures containing the growing plants; in others, strips of filter paper which had been saturated with the reagent were applied to the surface of growing roots. The substances used for showing the oxidizing power of growing roots were *α*-naphthylamine, benzidine, phenolphthalin, ferrous ammonium sulfate, Barbadoes aloes, guaiac, phloridzin, pyrogallol, leucomethylene blue, etc.

The extra-cellular oxidation by the roots of the phanerogams studied was found to be strongly localized and limited to the absorbing surface of the root. The most intensive oxidation occurs in the region covered by the root hairs. After the death of the root hairs,

[13] Sitzb. Akad. Wiss. Wien. Math. Nat. Kl. **96**:84. 1888.

[14] Jahrb. Wiss. Bot. **29**:321. 1896.

[15] Bull. Acad. Sci. Cracovie **1905**:338, 668, 693.

as the root grows older, the oxidation becomes weaker (as shown by the less intense coloration) and vanishes in basipetal order. The short growing zone of the root between the root cap and the region of root hairs shows very little if any oxidation. The cells of the root cap behaved differently in different plants. In some there was a very weak oxidizing power, insignificant in comparison with that of the absorbing region of the root; in other plants the root cap showed no power to oxidize. This observation is the more interesting because PFEFFER[16] regarded the experiments of MOLISCH to lack proof that the guaiac-bluing power was due to living cells and not to the dead or dying cells of the root cap.

The oxidation which occurred in naphthylamine and benzidine solutions first appeared on the outer surfaces of the walls of the root hairs and epidermal cells, later in the wall itself, and finally in the outer layer of the ectoplasm. When roots were left for a long time in a solution of these chromogens, the entire protoplasm of the epidermal cells and root hairs gradually assumed the dark color of the oxidized chromogen, although it was not determined whether this color was due to the diffusion inward of the dye formed at the surface, or to an actual intracellular oxidation.

MATERIAL AND METHODS

The experiments described in succeeding pages consisted in studying the oxidizing power of wheat plants grown under various conditions in connection with soil-fertility investigations. It was necessary to grow the wheat plants used for experimentation in solutions, since in such cultures it is possible to observe the oxidation without disturbing the roots. For the study of soil conditions an aqueous extract was made by stirring one part of soil with five parts of distilled water for three minutes and filtering after 30 minutes through a Pasteur-Chamberland clay tube. It has been found that soil extracts prepared in this manner possess a plant-producing power similar to that of the soil from which they were made. In other words, fertile soils yield extracts which promote good plant growth, and infertile soils give extracts producing poor plant growth.

The water used in making solutions and soil extract was the

[16] Abhandl. kön. säch. Gesells. Wiss. Leipzig, Math. Phys. Cl. 15:375. 1889.

ordinary laboratory distilled water treated with carbon black. The water was distilled from a copper boiler, condensed in a block-tin worm, and collected in a tin-lined copper tank. This method of distillation gives very good water for ordinary chemical work, but does not free it from traces of volatile organic compounds, which may exert a toxic action as was described by LIVINGSTON[17] and the writers.[18] It has been found that these deleterious substances may be effectually removed by treating the distilled water with some finely divided solid which possesses a strong absorbing power, such as ferric hydrate, or carbon black. The procedure usually followed was to shake up a small quantity of the carbon black in the water and let it stand for 30 to 60 minutes; at the expiration of that time the water was filtered through ordinary filter paper and was ready for use. This treatment has been found to be as efficient in producing physiologically pure water as redistillation with strong oxidizing agents, like acid potassium bichromate or alkaline potassium permanganate.

The varieties of wheat used in the experiments were "Chul" and "Harvest Queen." The seeds were germinated on floating perforated plates, according to the method described by LIVINGSTON[19] and in Bulletin 40 of this Bureau. The seedlings were transferred from the perforated germinating plates to the cultures just as the first true leaf was beginning to emerge from its sheath.

The plants were held in notches cut in the edge of a cork, as described by LIVINGSTON. In this way the seed in which enzymes were acting upon reserve food materials were kept out of the solutions and the enzyme effects observed were ascribable to substances arising from the roots.

Salt-mouth bottles, having a capacity of 250^{cc}, were used as culture jars, and ten wheat plants were grown in each jar. In each test two cultures containing 20 wheat plants were usually employed, and comparison was made with an equal number of plants growing in pure distilled water under the same conditions. All experiments were conducted in a greenhouse. During the season of the year

[17] Bur. Soils, U. S. Dept. Agr. Bull. 36. 1907.

[18] Bur. Soils, U. S. Dept. Agr. Bull. 40. 1907.

[19] Plant World **9**:13. 1906.

in which conditions were most favorable for growth, each experiment was conducted for 8 to 12 days before studying the oxidizing action of the plants, but during the cloudy winter weather the time was sometimes extended to 14 to 16 days.

In addition to determining the oxidizing power of the plants subjected to various treatments, their growth was estimated by recording the green weight and transpiration of each culture.[20]

SUBSTANCES CAPABLE OF SHOWING THE OXIDIZING POWER

Two classes of substances have been found useful in showing the oxidizing powers of plant roots in solution cultures. The first class comprises certain soluble chromogens, which yield, upon oxidation by the plant roots, insoluble colored compounds mainly deposited upon the surface of the roots. The oxidation is usually rapid enough to produce marked results before the surface extension of the roots perceptibly disturbs the zonal distribution of the colors. The second class of chromogens consists of certain substances which give soluble coloring matters as the result of the oxidizing action of the roots. The oxidizing action may be shown by the change from a colorless to a colored compound, or by a change from one color to another and distinctly different color.

Compounds belonging to the first class which have been used in this work are *a*-naphthylamine, benzidine, vanillin, vanillic acid, and esculin.

Alpha-naphthylamine is only slightly soluble in water, but constitutes a good reagent for use in plant cultures because its colorless solution is non-toxic, or nearly so, to plants. When oxidized by the roots of plants, or by reagents such as ferric chlorid or silver nitrate, *a*-naphthylamine is converted into the insoluble, lavender-purple oxynaphthylamine. When the oxidation is performed by the growing roots of a plant, the oxynaphthylamine is deposited upon the surface of the roots in characteristic zones, as already described by RACIBORSKI (*op. cit.* 357). The root cap is slightly if at all colored; the zone of primary meristematic cells immediately back of the root cap is marked by a distinct narrow band of color; the zone of actively

[20] For a discussion of the value of these criteria the reader is referred to LIVINGSTON, BOT. GAZETTE **40**:178. 1905; JENSEN, BOT. GAZETTE **43**:11. 1907; and Bureau of Soils, U. S. Dept. Agric. Bull. 47. 1907.

growing cells in the region of greatest elongation is not intensively colored; the more slowly growing portions of the root possess the purplish color, but it becomes less intense as one passes to the upper parts of the root.

The superior oxidizing power of the meristematic tissues of the plant is not only shown by the narrow zone of deep color formed on the primary meristem of the apical portion of the roots, but also by the small dots of color produced on that portion of the root from which secondary roots arise. If a wheat root 8 to 10^{cm} in length is placed in a solution of naphthylamine it will exhibit, in addition to the deeper colored zones near the apex, dark-purple spots at the places where secondary roots are forming and are about to break through the cortical layers of the primary root. If secondary roots are already present they show the same zones of colors already described for the primary roots.

The concentrations of naphthylamine used in solution cultures are necessarily low on account of its slight solubility in water, but are sufficiently strong to show the oxidation. In ordinary practice 10 parts of naphthylamine to a million (10^{mg} per liter of water) is a suitable concentration to use. This concentration will eventually retard the growth of wheat plants, but is not detrimental to growth in the length of time usually required to demonstrate the oxidizing powers of the plant roots. A concentration of 5 parts per million sometimes acts as a stimulant to growth.

Benzidine is another chromogen, which is oxidized by plants and may advantageously be used to demonstrate their oxidizing action. It is only slightly soluble in water, but in weak, colorless solution it is readily oxidized by plant roots to an insoluble dye which gives the roots a blue-black or black appearance. Benzidine is slightly toxic to plant growth, but does not cause pathological conditions within the time required for demonstrating the oxidizing power of the plant roots. A concentration of 5 parts benzidine to a million of water will give good results and does not injure wheat roots in 24 hours, although that concentration may eventually inhibit growth.

The effect of oxidation may easily be demonstrated by allowing the roots of wheat plants to grow in a 5 parts per million solution of

benzidine for 12 to 24 hours. The formation of colors in distinct zones is fully as striking as in the case of α-naphthylamine. As before, the root cap does not produce oxidation products, the primary meristem is marked by a narrow band of brown color, the zone of elongation is practically uncolored, whereas the portion of the root just above the zone of greatest elongation is entirely colored blue-black or black by the oxidation products.

Solutions of vanillin and vanillic acid act in much the same manner as those of naphthylamine or benzidine, but the concentrations required to demonstrate the oxidizing power of roots are quite strongly toxic.[21] Both substances are converted by the oxidizing action of the roots into a purple insoluble dye which stains the surface of the roots in the manner previously described. The concentration of vanillin in the solution most favorable for showing oxidation with wheat plants lies between 250 and 500 parts per million. A solution of this concentration will demonstrate the oxidizing power of the roots before the plants become seriously injured. To demonstrate the oxidizing power of roots with vanillic acid, a solution of the latter containing 25 to 50 parts per million should be used.

Esculin is another chromogen belonging to this class, but was found to be less suitable for this work. Esculin solutions, when freshly prepared, exhibit a blue fluorescence. After plant roots have grown for a few days in such a solution, the blue fluorescence is lost, and the roots themselves are colored yellow as a result of their oxidizing activity, the dye formed being insoluble and remaining upon the surface of the roots where the greatest oxidation occurs. The concentrations necessary to demonstrate the oxidizing power of roots range from 500 to 1000 parts per million, and are eventually quite toxic to wheat plants.

The second class of chromogens, viz., those which are converted into soluble coloring matters, are in many respects more useful for oxidation studies than those belonging to the first class, because the intensity of the color, and hence the amount of oxidation, can be quantitatively expressed. The substances belonging to the second class which have been employed in this study are phenolphthalin,

[21] Bur. Soils, U. S. Dept. Agric. Bull. No. 47. 1907. Proc. Soc. Biol. Chem 1:33. 1907. BOT. GAZETTE 45:73. 1908.

aloin, and leuco-rosolic acid. Alcoholic solutions of guaiac were also used for various tests, but could not be put into solution cultures containing growing roots.

The value of phenolphthalin, a leuco-compound prepared from phenolphthalein, as an indicator of oxidizing enzymes, has been demonstrated for plant work by KASTLE[22] and by RACIBORSKI.[23] Phenolphthalin is prepared by the method described by BAEYER,[24] which consists in reducing ordinary phenolphthalein with zinc dust and sodium hydroxid to phenolphthalin. The latter substance is oxidized back to phenolphthalein by the oxidizing power of the plant roots, a change which is readily demonstrated when the solution is rendered alkaline. The following procedure was observed in preparing this reagent. Weigh out 250^{mg} of phenolphthalein, 3^{gm} of sodium hydroxid, and 4 or 5^{gm} of zinc dust. Place all in a flask and add 100 to 150^{cc} of water. Place the flask on a sand bath and heat sufficiently to cause a rapid evolution of hydrogen, without causing the contents of the flask to boil violently. The heating usually requires 2 to 3 hours to effect reduction of the phenolphthalein. The contents of the flask, after reduction is completed, may be filtered and rendered nearly neutral with hydrochloric acid, and may then be used as an indicator in the plant cultures. However, better results may be obtained by using phenolphthalin purified according to the method given by BAEYER. After purification the phenolphthalin is dissolved in $N/10$ or $N/20$ NaOH and a few cubic centimeters of the alkaline solution put into each culture, adding equal amounts to cultures which are to be compared. If quantitative results are desired, it is necessary to reduce all the solution cultures to neutrality or the same degree of alkalinity. A very slight degree of alkalinity is not usually harmful to plants within the duration of an experiment, and is favorable to the process of oxidation. Phenolphthalin is slowly oxidized by mere contact with the air; therefore it is advisable to instal controls which will allow the results to be corrected for this atmospheric oxidation. When the phenolphthalin is added to the solution cultures, a like quantity is therefore added to jars of distilled

[22] Amer. Chem. Jour. **26**:526. 1901. Hyg. Lab. U. S. Pub. Health and Mar. Hosp. Ser. Bull. 26. 1906.

[23] Bull. Acad. Sci. Cracovie, Math. Nat. Cl. **1905**:338.

[24] Annalen Chem. **202**:80. 1880.

water equal in volume to the cultures. The amount of oxidation in these blanks is subtracted from what is observed in the plant cultures.

Plant cultures usually show striking results at the end of 10 to 20 hours, depending somewhat upon the temperature and amount of root surface. At the end of the experiment the plants are removed from the cultures, and all are rendered distinctly alkaline with sodium hydroxid solution, and thus the red phenolphthalein color appears.

The great advantage in the use of phenolphthalin to demonstrate the oxidizing power of roots lies in the fact that it is capable of yielding quantitative results. After the colors have been developed in the alkaline solution, their intensities may be estimated by the aid of a colorimeter. In the work reported below the color intensities were estimated by means of the colorimeter previously described,[25] which permits of rapid and accurate readings. The colored solutions may be read against a standard phenolphthalein solution or against a standard Lovibond red glass slide.[26] The readings of the colorimetric tubes are inversely proportional to the color intensity and are easily reduced to their relative values.

Aloin is a substance which may be used to demonstrate the oxidizing power of roots in the same way as phenolphthalin is used. Aloin, or barbaloin, is the active principle of Barbadoes aloes, and is obtained in the market in the form of a yellow powder, fairly soluble in water and serving well as an indicator of the oxidizing power of plants. At the concentrations used in our work it was not found to exert any toxic action upon plants. As a result of a limited investigation of the chemistry of aloin, it seems that its value as an indicator of the oxidizing power of plants depends largely upon the content of iso-barbaloin.

When oxidized by the plant roots, the aloin solution is changed from a pale yellow color to a permanent deep wine-red color, similar to that given by KLUNGE'S reaction for iso-barbaloin. KLUNGE'S reaction[27] consists in dissolving aloin (containing iso-barbaloin) in

[25] Jour. Amer. Chem. Soc. **27**:1192. 1905. Bur. Soils, U. S. Dept. Agric. Bull. 31, 1906.

[26] LOVIBOND, Jour. Soc. Chem. Ind. **13**:308. 1894; see also SCHREINER, Pharm. Rev. **19**:61. 1901.

[27] Schweizerische Wochenschr. Pharm. **21**:1. 1883; also Leger, Compt. Rend. Acad. Sci. Paris **131**:55. 1900.

a 15 per cent. sodium chlorid solution and adding 5^{cc} of concentrated copper sulfate solution. Almost immediately the straw-yellow solution begins to change to a permanent deep wine-red. The change is hastened by warming the solution.

When experimenting with plant juices containing enzymes, there appears to be a difference between the reactions to aqueous and alcoholic solutions of aloin. As the result of experiments described in detail in a subsequent section of this paper, it was found that an aqueous solution of aloin is a better indicator of the presence of oxidase, while an alcoholic solution of aloin is the better indicator of peroxidase.

Aloin, like phenolphthalin, should be added to neutral or faintly alkaline culture solutions, and where quantitative results are desired all solutions should be of the same degree of alkalinity. In all of our work aloin was added at the rate of 100^{mg} of aloin to 250^{cc} of culture solution. If actively growing seedlings are used in a very faintly alkaline solution, a small amount of red color may be developed in an hour or two, but the experiments should be continued for 12 to 20 hours for the final observation. When certain inorganic salts were present in the culture solutions, the aloin red color was slightly modified. The addition of nitrates or previous treatment of the soil extracts with an absorbing agent gave the oxidized aloin a purplish tinge, resembling that of fresh fuchsin solution. The presence of calcium carbonate gave a purer red color, resembling alkanna or cochineal solution.

The fact that aloin is changed by oxidation from a light yellow to a deep red solution makes it somewhat more difficult to obtain colorimetric readings than in the case of phenolphthalin, where there is a change from a colorless to a red solution. Nevertheless, it is practical to use the colorimeter for measuring approximately the intensity of color in aloin solutions, by arranging the solutions in the order of their apparent color intensities, and using each solution first as an unknown and then as a standard for the next higher. For example, let No. 1, the weakest color, be the standard against which No. 2 is read. Then discard No. 1; set No. 2 at a convenient mark, and, using it as the standard, read No. 3. In turn No. 3 is used as the standard for No. 4, and so on. In this way one avoids

the necessity of comparing a solution strongly tinged with yellow against a solution which contains little or no yellow tint. In any two solutions to be estimated the tints of yellow should not be greatly different.

Leuco-rosolic acid is another reagent which is useful for demonstrating the oxidizing power of plant juices[28] and plant roots. When a few cubic centimeters of a slightly alkaline, colorless solution are added to a culture containing plants, the leuco-rosolic acid is oxidized back to rosolic acid, the change being shown by the appearance of the red color. This reagent is not so generally useful as phenolphthalin and aloin, since it is more readily oxidized by mere contact with the air, as well as being more difficult to prepare.

PRELIMINARY EXPERIMENTS

The first experiments were conducted for the purpose of ascertaining some general facts concerning the phenomenon of oxidation by the roots of seedlings, as well as to learn the methods best suited for studying oxidation in soil extracts. The experiments of RACIBORSKI dealt with plants growing under what may be termed pure culture conditions, and those of KASTLE were concerned with the oxidizing power of plant extracts.

In the first experiment, wheat seedlings 4 days old were placed in solutions of *α*-naphthylamine having concentrations of 1, 2, 5, and 10 parts per million, and in a solution of 5 parts per million benzidine. The experiment was set up at 4 P. M. August 6, and observations were made eighteen hours later. At the expiration of that time colors could be distinctly seen on the white surface of the wheat roots. The roots in the solution of 1 part per million naphthylamine were pale lavender; in 2 parts per million they were pronounced lavender, except at the root cap; in the 5 parts per million solution they were violet in the region occupied by the primary meristem, and in the region of the root hairs where growth of elongation occurs, while the root cap and a narrow zone just above the primary meristem were uncolored; in the 10 parts per million solution the roots showed the same colors as in that of 5 parts per million. The roots in the solution of 5 parts per million of benzidine showed their

[28] KASTLE, J. H., Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv. Bull. 26: 17. 1906.

power of oxidation by the formation of brown-violet color, distributed in the same manner as described for the roots which grew in the solutions of naphthylamine.

In order to learn whether the oxidizing powers of roots were affected by conditions which favor growth, and also whether the method used in the first experiment would show such differences, the following experiment was made. Three water cultures were made, in each of which an equal number of wheat seedlings of uniform age and size were employed. One culture was made with redistilled water, the second with an aqueous extract of a rich garden soil, the third with a dilute aqueous extract of well-decomposed stable manure. After the plants had grown for one day in these liquids, the oxidizing powers of the plants were observed by transferring them to other bottles containing 2 parts per million of *a*-naphthylamine in distilled water. At the expiration of 18 hours the intensity of the purple colors showed that the roots which had previously grown in the extract of garden soil had oxidized more naphthylamine than those which had grown in distilled water, and those which had grown in manure extract had oxidized more naphthylamine than those from the garden soil extract. At the end of 24 hours the differences in color intensity in the two cultures were still more marked.

The next experiment was an attempt to employ a method which would permit a more accurate quantitative expression of the oxidizing power of the roots. Two cultures of wheat seedlings were grown for 5 days in an extract of unproductive soil, under the same conditions as two other cultures in an extract of rich garden soil. Each culture contained 60^{cc} of the respective soil extract. The oxidizing power of the roots in this experiment was shown by using phenolphthalin. The phenolphthalin was prepared by the method given in a previous paragraph, and 0.4^{cc} of the freshly prepared solution were added to each culture of plants after they had grown 5 days in their respective solutions. Nineteen hours after adding the indicator all plants were removed from the cultures and the solutions rendered alkaline, thus producing the phenolphthalein color. The solutions were brought to the same volume by the addition of distilled water, and the relative amount of oxidation was measured by determining the color intensities of the different cultures.

The two cultures of poor soil gave readings of 40 and 42 divisions on the graduated tube against slide No. 2 (Lovibond system); the two cultures of rich garden soil gave readings of 14 and 24 divisions against slide No. 4 (Lovibond system). Averaging the readings and comparing the intensity of the colors, the oxidation in the poor lawn soil and in the rich garden soil stands in the ratio of 1 to 4, or more exactly as 19 to 82. This result indicated that a procedure based upon this method will give satisfactory quantitative results.

This method was further tested by another experiment in which different beneficial treatments were applied to an extract of the unproductive soil used in the last experiment. The results of the last experiment showed that the oxidizing powers of plants growing in solutions of different physiological properties vary considerably, but left the question open as to how much of the oxidation result might be due to plants and how much to the solution. In the present experiment, therefore, two of the four bottles in each set of solutions were left unplanted, and their oxidizing powers measured along with those of the solutions which contained plants. The treatment employed consisted in adding fertilizer substances in the form of pure chemicals. Calcium carbonate was added at the rate of 2000, and sodium nitrate at the rate of 50 parts per million. The cultures were put up August 24 and allowed to grow until August 28, when the amount of water transpired by each culture was ascertained and 3^{cc} of a freshly prepared phenolphthalin solution added to each bottle. The color of the phenolphthalein was brought out by adding a few drops of strong alkali to each culture, and the intensities of the different solutions were compared in the colorimeter. Table I presents the figures which give the relative amount of oxidation in the planted and unplanted solutions. When the phenolphthalin solution was added to the culture jars, the same quantity was added to a jar of distilled water, which served as a control upon the oxidation incident to contact with atmospheric oxygen. The color intensity of the control was determined and subtracted from each of the other readings.

The plants used in this experiment were quite young, and the experiment was only continued for four days, a period rather too short for the maximum oxidation effect, as shown by subsequent experiments; nevertheless, the results show that the different treatments

TABLE I

Relative oxidizing power of cultures and unplanted solutions of Takoma lawn soil extract with and without the addition of fertilizer ingredients. Oxidizing power of plants grown in distilled water used as the basis of comparison (p.p.m. = parts per million).

No.	Solutions	Relative oxidation
1	Distilled water (planted) } Average	100
2	Distilled water (planted) }	
3	Extract Takoma lawn soil (planted)	88
4	" " " " "	74
5	" " " " (unplanted)	8
6	" " " " "	19
7	" " " " +2000 p.p.m. $CaCO_3$ (planted)	113
8	" " " " +2000 " " "	63
9	" " " " +2000 " " (unplanted)	19
10	" " " " +2000 " " "	25
11	" " " " + 50 p.p.m. $NaNO_3$ (planted)	98
12	" " " " + 50 " " "	63
13	" " " " + 50 " " (unplanted)	24
14	" " " " + 50 " " "	17

affected the oxidizing powers. The plants grown in extracts of poor soil possessed less oxidizing power than the controls in distilled water, but the oxidizing power was increased by the addition of calcium carbonate. The addition of sodium nitrate did not show any marked increase to the oxidation in those solutions in the time of the experiment, although its effect as shown in later experiments is always beneficial to oxidation.

The point to be emphasized in this experiment, which has not been previously brought out, is that the soil extract unplanted possesses a comparatively feeble power of oxidation, as shown by the use of phenolphthalin, and that the addition of calcium carbonate and sodium nitrate slightly increased this small oxidizing power.

An additional experiment was performed, using three different salts in distilled water. The results of this experiment, which are given in Table II, confirm those of the foregoing experiment in the soil extract. The cultures were made in duplicate and the figures represent the averages of each pair.

The enzymotic nature of the oxidizing processes was next investigated, using alcoholic guaiac. When alcoholic guaiac is added to a solution in which wheat roots have been growing for a time, evidence

of the presence of peroxidase was obtained, but not of oxidase; however, when young growing wheat roots are treated with a solution of alcoholic guaiac they instantly give a blue color, which deepens when hydrogen peroxid is added. This indicates that the cells of the plant root contain an oxidase, as CZAPEK has shown.[29]

TABLE II

Relative oxidizing power of cultures and unplanted solutions of three nutrient salts. Oxidizing power of plants grown in distilled water used as basis of comparison (p.p.m. = parts per million).

No.	Solutions	Relative oxidation
1	Controls in distilled water (planted)	100
2	Solution, 50 p.p.m. of NO_3 as $NaNO_3$ (planted)	282
3	" 50 " " " " (unplanted)	39
4	" 35 " K as KCl (planted)	72
5	" 35 " " " (unplanted)	36
6	" 50 " PO_4 as Na_2HPO_4 (planted)	88
7	" 50 " " " (unplanted)	21

A word may be introduced at this place concerning the possible function of bacteria in producing oxidizing ferments which might accomplish some of the effects noted. It is, of course, possible that such organisms existed in the culture employed, since after filtering the extracts no especial precautions were taken to keep them sterile, and microorganisms which were on the roots of the plants would certainly be introduced into the solutions. That these microorganisms were responsible for any appreciable amount of oxidation in the experiments described in this paper is hardly possible. In the first place, the solutions used were not well adapted for a very thrifty development of microorganisms, as was shown by their freedom from turbidity, odors, or other indications. The definite zones of color produced when indicators like *a*-naphthylamine and benzidine were used, and their close correspondence to definite zones of tissue in the root show that the oxidation is performed only by agents intimately connected with the roots. The colors due to oxidation were most intense on the regions of the root where growth was most active, whereas we would expect that the bacteria, if zonally distributed, would be more abundant on the dying cells of the root cap

[29] Annals of Botany **19**:75. 1905.

or on the dismantled cortical layers of the older parts of the root. It seems, therefore, highly improbable that the oxidizing activities of microorganisms can be responsible to any appreciable extent for the results observed.

OXIDATION IN SOIL EXTRACTS

Following the preliminary experiments already described, further experiments were made to study in more detail the oxidizing power of plants grown in extracts of soil of different character. These experiments were chiefly designed to study the oxidizing powers of plants in extracts of good and poor soils, of extracts treated with absorbing agents, and in distillates of soil extracts.

The difference in oxidizing power of plants in extracts of fertile and infertile soils is shown by the following experiments. In the first experiment, an extract of Takoma lawn soil was compared with an extract of good Leonardtown loam. The former is a very unproductive soil, and the latter is a much better and usually a very productive soil. The oxidizing powers of the plants were determined by adding phenolphthalin to the cultures, after the plants had grown in them for nine days. The growth and oxidizing powers of the plants are shown in Table III, relative to control cultures made in distilled water, which are represented as 100 in each case.

TABLE III

Comparative growth and oxidizing powers of plants in extracts of Takoma lawn soil and good Leonardtown loam. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1	Controls in distilled water	100	100
2	Extract Takoma lawn soil	33	72
3	Extract Leonardtown loam	50	286

In the comparatively short time of this experiment during cloudy winter weather, December 10 to 17, the plant growth as manifested by the figures for transpiration did not have time enough to show the relative productiveness of the two extracts, since it has usually been found that the Leonardtown loam extract produces in 14–18 days better plants than distilled water. The figures do show, however,

a much greater oxidizing power in the plants grown in the extract of the more fertile soil, even under these conditions.

Subsequent experiments were performed, the results of which corroborated the foregoing. In each case where growth was good, there was also good oxidation; where growth indicated a poor soil extract, the oxidation was small, as will be seen from Tables IV and V.

TABLE IV

Comparative growth and oxidizing powers of plants in extracts of poor sandy loam and garden loam. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1.....	Controls in distilled water	100	100
2.....	Extract poor sandy loam	77	103
3.....	Extract garden loam	125	275

TABLE V

Comparative growth and oxidizing powers of plants in extract of good and poor oils. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1.....	Controls in distilled water	100	100
2.....	Extract Arlington clay loam	75	107
3.....	Extract Clarksville silt loam	123	133
4.....	Extract Stockton peat	272	400

In all these experiments where direct comparisons are made between the extracts of soils which were so poor as to give less plant growth than pure distilled water, and other extracts giving materially greater growth than the same, it appears to be unmistakably true that the cultures made in extracts of good, fertile soils possess much greater oxidizing powers than those made in extracts of soils of relatively less fertility.

The next question considered was concerned with the effect of treating the soil extract with absorbing agents. Treating the extracts of a more or less unproductive soil with carbon black or other good absorbing agent is usually beneficial to growth. This response seems to be quite general for all poor soil extracts, although their response to other treatments may be quite different. Previous work in this

laboratory[30] has shown that this ameliorating action is due to the removal of deleterious organic substances. Extracts were treated with carbon black or ferric hydrate. The absorbing agent was shaken with the soil extract and filtered off at the expiration of a half-hour, in the same manner as the distilled water used in the experiments was prepared. The relative effects of this treatment upon growth and the oxidizing power of the plants is shown in Table VI, where the effect in the untreated soil extract is in each case taken as 100.

TABLE VI

Effect of treatment with carbon black and ferric hydrate upon growth and oxidizing power of plants grown in extracts of various soils. Growth expressed in terms of relative transpiration.

No.	Soil extract	Relative growth	Relative oxidation
1	Arlington clay loam*	100	100
	" " " carbon black treated	124	265
2	Takoma lawn soil*	100	100
	" " " carbon black treated	137	100
3	Alloway clay*	100	100
	" " carbon black treated	116	117
4	Dunkirk sandy loam*	100	100
	" " " carbon black treated	112	280
5	Miami silt loam*	100	100
	" " " ferric hydrate treated	171	198
6	Marshall clay loam†	100	100
	" " " carbon black treated	216	130
7	Clarksville silt loam†	100	100
	" " " carbon black treated	450	227
8	Elkton silt loam†	100	100
	" " " carbon black treated	179	317
9	Cecil fine sandy loam†	100	100
	" " " " carbon black treated	112	200
10	Hagerstown loam†	100	100
	" " carbon black treated	230	500
11	Cecil sandy loam†	100	100
	" " " carbon black treated	193	241
12	Dutchess silt loam†	100	100
	" " " carbon black treated	110	373
13	Poor sandy loam†	100	100
	" " " ferric hydrate treated	170	534
14	Garden loam†	100	100
	" " ferric hydrate treated	136	313

* Phenolphthalin used in estimating oxidation.
† Aloin used in estimating oxidation.

It will be noted that in all but one of the soil extracts the effects of the treatment with an absorbing agent strongly increased the

[30] Bur. Soils, U. S. Dept. Agric. Bulls. 28 36, 40. Jour. Amer. Chem. Soc. **30**: 1295. 1908.

oxidizing powers of the plants subsequently grown in the extracts, and the growth of the plants was also increased.

The increased oxidation, as well as the increased growth, points directly to the conclusion that the soil extracts have been so improved by the treatment given as to induce a more active functioning of processes necessary to secure the best conditions for growth. In the single case of No. 2 the growth was increased as a result of the treatment with carbon black, but the oxidation was not. This result was frequently obtained with the Takoma lawn soil; in some cases the oxidizing power was even slightly decreased as a result of treatment with absorbing agents, although growth was increased. No satisfactory explanation has as yet been obtained for this apparently exceptional action. It may be found upon further investigation that the lack of response was due to the presence of matter inhibiting oxidation, which was not removed by the carbon black. This question seems worthy of more study than we have been able to give it.

Extracts of poor soils sometimes contain volatile bodies of a deleterious nature, which can be driven off by boiling and collected in the distillate. The writers have described[31] the behavior of plants grown in such distillates. Where the deleterious bodies are volatile, the distillate usually exhibits the same toxic properties which the original extracts previously possessed, and the residue is correspondingly improved.

To study the effects of these distillates upon the oxidizing powers of the plants the following experiments were made. One liter of such soil extract was placed in a distilling apparatus and distilled until 200^{cc} of distillate had passed over and been condensed. This fluid was made up to 500^{cc} by adding water and designated first portion. When a second 200^{cc} of distillate had been collected, it was likewise made up to 500^{cc} and designated second portion. Cultures were made in each portion, together with controls in pure distilled water. At the end of a week the plants in the different solutions showed marked differences. The plants in the first portion of the distillate were very small and were dying; those in the second portion were much better, in fact, were equal to the controls growing

[31] Bur. of Soils, U. S. Dept. Agric. Bull. 40. 1907.

in distilled water. One hundred milligrams of aloin was added to each of the culture bottles, and on the following day the amount of oxidation was noted by comparing the intensity of red color in each culture. The cultures in the first portion showed much less oxidation than either of the other two. The most oxidation appeared to have gone on in the cultures in the second portion, which was slightly in excess of that in the control cultures in pure distilled water.

The question was studied further, and in a more quantitative manner, by the following experiment. An extract of Elkton silt loam, having a volume of 750^{cc}, was placed in a flask connected with a condenser and distilled. The distillate, amounting to 500^{cc}, was collected in two portions of 250^{cc} each and used as a culture medium in which plants were grown. The residue in the distilling flask, which was diluted to its original volume, was also used for growing plants. For comparison, cultures were also made in the original soil extract. The wheat plants were allowed to grow in the various solutions for 13 days, and then their oxidizing powers were estimated by means of phenolphthalin. The growth and oxidation are shown in Table VII.

TABLE VII

Growth and oxidation in distillate and residue of extract from Elkton silt loam. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1.....	Original soil extract untreated	100	100
2.....	First portion of distillate	53	20
3.....	Second portion of distillate	70	19
4.....	Residue after distillation, diluted to original volume	132	180

These results show that the distillates of this soil extract were less favorable for growth and oxidation than the original untreated soil extract, while the residue from distillation was materially improved. This seems to indicate that the original soil extract, like others which have been investigated,[32] contained a volatile toxic substance which inhibited oxidation by the roots, and that this substance was driven off by the process of distillation, with resulting

[32] Bur. Soils, U. S. Dept. Agric. Bulls. 28, 36, and 40.

benefit to oxidation in the residue. Judging from the growth of the plants, the first portion of the distillate contained a larger proportion of this deleterious substance than the second, although this smaller amount appears to be just as deleterious to the oxidizing powers of the roots as the larger amount present in the first portion. The oxidizing power of the plants in the residue was much greater than in the distillates or in the original soil extract.

Evidently the oxidizing powers of the roots are affected by certain external conditions, since an improvement in the physiological properties of the soil extract results in increased oxidation, and the presence of deleterious bodies results in decreased oxidation.

From the experimental results thus far presented, it appears that the oxidizing power of the soil extracts themselves can be regarded as partly, but not mainly, responsible for the oxidation observed in the experiments. In one of the preliminary experiments reported in Table I, it was shown that the soil extract after filtration through a Pasteur-Chamberland filter tube exhibited some oxidation, even when no plants were growing. It was likewise shown by the results in Table II that certain nutrient salts dissolved in distilled water were able to accomplish a material amount of oxidation without the presence of growing plants. It seems unlikely, therefore, that any considerable amount of oxidation was performed by microorganisms. If we consider the result in this last experiment, where oxidation was increased in the residue from distillation after continued boiling, it seems that any extensive action, not only of microorganisms, but also of enzymes, must be precluded. In the soil, however, it is quite probable that both of these oxidizing factors would come into play, but it is quite certain that the oxidizing power of the roots would accomplish a considerable portion of the oxidation observed.

THE NATURE AND ACTIVITIES OF THE OXIDIZING ENZYMES

Mention has been made in preceding pages of the enzymotic nature of the oxidizing action of the roots and consideration will now be given to the nature of the enzyme or enzymes which bring about the oxidation. So far as known, the oxidation effects observed were entirely due to the action of enzymes and not to the other activities connected with the growth of the roots themselves.

When a few drops of alcoholic guaiac are added to water, or a suitable solution of salts, in which wheat seedlings have grown for several days, there is sometimes a faint blue color, indicating the presence of oxidase, but more often there is no blue color. When a drop of hydrogen peroxid is added, however, the liquid turns blue, giving a color varying from medium to very intense, depending somewhat upon the age of the seedlings, and the number of roots which have grown in the culture. The guaiac-peroxid reaction indicating a peroxidase is confirmed by the reaction to phenolphthalin and aloin, both of which agree in showing the presence of peroxidase. When the roots of a young wheat plant are immersed in an alcoholic guaiac solution, they immediately turn blue, indicating that they are relatively rich in oxidase, although but little oxidase appears in the water in which they grew. This may be due to the retention of oxidase by the root cells during life, but when the outer cells are killed by the alcoholic guaiac the oxidase escapes and becomes evident through its reaction with guaiac. An aqueous extract of crushed roots, shows strong oxidase reaction as well as peroxidase reaction. In the course of a brief examination of different parts of the young wheat plants, it was found that the partially depleted seeds showed a very strong oxidase reaction when guaiac was used, while the peroxidase reaction was relatively less than in the extract of crushed roots.

When the solution in which wheat roots have been grown for some days is boiled for five or ten minutes, and cooled, the oxidase and peroxidase reactions disappear.

The temperature at which the peroxidase is destroyed was determined by heating a culture liquid which showed an active peroxidase action. The culture liquid was heated to successively higher temperatures and held at each for five minute periods. The temperature at which the enzymes appeared to be destroyed was 60° C. or very close thereto.

The culture liquid was examined for enzymes in a series of cultures of different ages to learn whether the enzyme reaction was equally strong in all. Wheat seeds were germinated on perforated cork plates floating on the surface of water in crystallizing dishes of 500^{cc} capacity. When cultures were on hand aged two, three, four, five,

six, and seven days respectively, tests were made with guaiac, alcoholic aloin, and phenolphthalin.

The tests with guaiac showed that the oxidase reaction which was weak in the two- and three-day cultures was quite strong at four days. The tests with alcoholic aloin and phenolphthalin showed that the peroxidase reaction was strongest in the six- and four-day cultures, and considerably weaker in each of the others.

Certain phenomena observed in connection with the use of aloin in aqueous and alcoholic solutions, suggested that they react differently with oxidases and peroxidases. Experiments were accordingly installed to test specifically the action of each solution. Two solutions of aloin were prepared: I, 0.250^{gm} of aloin in 50^{cc} of water; II, 0.250^{gm} of aloin in 50^{cc} of 95 per cent. alcohol. One cubic centimeter of aloin solution I or II was added to 5^{cc} of liquid in test tubes, according to the plan shown in Table VIII. The tubes were prepared and aloin added at 2.45 P. M., on January 10, and the observations recorded in the third column of the table were made at 11 A. M. on January 11. The culture liquid when added to the tubes showed no oxidase but good peroxidase reaction with guaiac.

TABLE VIII

Comparative reaction of aqueous and alcoholic solutions of aloin to a liquid containing peroxidase.

Nos.	Solution	Color observed 11 A. M., January 11
1 and 2.....	Unboiled liquid + 1^{cc} aqueous aloin	pink
3 and 4.....	Boiled " + " "	pink
5 and 6.....	Distilled water + " "	faint pink
7 and 8.....	Unboiled liquid + alcoholic "	deep pink
9 and 10.....	Boiled " + " "	yellow
11 and 12.....	Distilled water + " "	yellow

An inspection of these results shows that when only peroxidase is present, aqueous aloin is not particularly applicable for demonstrating the presence of that enzyme in the absence of growing plants, since there was the same development of pink color in the boiled as in unboiled liquid. Alcoholic aloin, on the contrary, was changed to a deep pink in the unboiled liquid, but remained unchanged alike in the boiled liquid and in the distilled water.

The action of the different aloin solutions was next tested in liquids

which also possessed a strong oxidase reaction. The roots of 10 wheat seedlings 12 days old were removed and crushed in a mortar with distilled water. The filtered liquid obtained from this source gave a strong reaction for oxidase when tested with guaiac. As before, 1^{cc} of aloin solution I or II was added to 5^{cc} of the root extract in test tubes, according to the plan in Table IX. The tubes were prepared and aloin added at 4.30 P. M. on January 13, and the observations recorded in the third column of the table were made at 11 A. M. on the following day.

TABLE IX

Comparative reaction of aqueous and alcoholic solutions of aloin to a liquid containing oxidase.

Nos.	Solution	Color observed 11 A. M., January 14
1–2–3	Root extract + 1^{cc} aqueous aloin	red
4 – 5	Distilled water + 1^{cc} aqueous aloin	faint pink
6–7–8	Root extract + 1^{cc} alcoholic aloin	pronounced pink
9 –10	Distilled water + 1^{cc} alcoholic aloin	faint pink

The results of these experiments supplement those of the foregoing in which a peroxidase liquid was used, by demonstrating that the oxidase caused a much greater conversion of aloin to "aloin red" with the aqueous than with the alcoholic solutions of aloin. There was in the root extract a distinct peroxidase reaction to guaiac, in addition to the oxidase reaction, and it is therefore only natural that in tubes 6, 7, and 8 there should be some development of color when alcoholic aloin was added.

It is evident, from the above results, that in the absence of living plant roots aqueous aloin is principally a reagent for oxidase and alcoholic aloin for peroxidase. In the experiments where plants are employed it is however needless to say that only aqueous solutions of aloin can be used.

Aloin and phenolphthalin having shown their usefulness as indicators of enzyme action, several other substances were investigated for comparison. Leuco-rosolic acid was prepared by reducing rosolic acid with zinc dust in alkaline solution. When reduction was practically complete, the solution was filtered and neutralized with hydrochloric acid, then rendered slightly alkaline with sodium

hydroxid. One cubic centimeter of this solution was added to three different liquids: I, liquid from culture 6 days old; II, the same liquid after having been boiled 10 minutes; III, distilled water. When examined 24 hours later I was pronounced rose-red, while II and III were merely faint pink; which indicates that leuco-rosolic acid is capable of showing the action of those oxidizing enzymes.

Attempts were made to use ferrous ammonium sulfate and potassium iodid as indicators of the oxidizing powers of plants by putting small amounts into cultures containing living plants. Ferrous ammonium sulfate was, in the space of time of the experiment, oxidized by mere contact with the atmospheric oxygen, and was therefore discarded as an indicator. Potassium iodid was not oxidized to free iodin, as RACIBORSKI has also found.[33]

EFFECT OF DIFFERENT CONDITIONS IN THE SOLUTIONS UPON THE ACTIVITY OF THE ENZYMES

Mention has previously been made of instances where the variation in oxidation appeared to be partly due to the acidity or alkalinity of the solution used as a culture liquid. In such cases the growth of the plant roots was affected whenever the alkalinity or acidity was very pronounced. The effect is the more harmful when young seedlings are put into such solutions, because at the beginning of the experiment, when the plants are very tender, the acidity or alkalinity is greatest and gradually diminishes during the progress of the experiment. In investigating the effect of acid or alkaline conditions in the the culture media, instead of using either alkaline or acid solutions at the start, a method was used whereby the originally neutral solutions became acid or alkaline as a result of the selective absorption of the plant in withdrawing nutrients from the solution.[34] It has been demonstrated by KOHN and CZAPEK[35] that fungi may render their culture media alkaline or acid as a result of their selective absorption, whereby an acid or a basic radical is removed more rapidly than the radical to which it is linked. REED[36] has observed a similar action

[33] Bull. Acad. Sci. Cracovie **1905**:668.

[34] See CAMERON, Rept. U. S. Dept. Agr. **71**:67. 1902; Bur. Soils, U. S. Dept. Agric. Bulls. 30 and 41. 1905.

[35] Beitr. Chem. Phys. Path. **8**:302. 1906.

[36] Annals of Botany **21**:501. 1907.

for the higher plants and pointed out its bearing upon the composition of nutrient solutions.

Solutions were made up from salts whose radicals are differently absorbed by growing plants, e. g., calcium nitrate and potassium sulfate. Where calcium nitrate is furnished the plant takes up NO_3 more rapidly than Ca, with the result that the solution becomes increasingly alkaline. In the case of potassium sulfate, the plants take up K more rapidly than SO_4, with the result that the solution becomes acid. In the experiments which were made upon this problem, an attempt was made to determine the acidity or alkalinity of the solutions when the experiment was terminated. A measured quantity of solution was boiled in a platinum vessel to drive off CO_2, and then titrated. The results of these determinations are shown with the other results in Table X.

TABLE X

Oxidation and growth of wheat plants in solutions which became acid or alkaline as the result of plant growth. Relative growth measured by transpiration.

No.	Solution	Relative growth	Acidity at termination of the experiment	Alkalinity at termination of the experiment	Relative oxidation
1.	Control in distilled water	100	.....	.....	100
2.	30 p.p.m. Ca as $Ca(NO_3)_2$	174	.....	$n/5000$	401
3.	30 p.p.m. Ca as $CaCl_2$	112	.....	$n/7500$	107
4.	30 p.p.m. Ca as $CaCO_3$	123	.....	$n/7500$	175
5.	66 p.p.m. SO_4 as $(NH_4)_2SO_4$	46	$n/7500$	.....	102
6.	66 p.p.m. SO_4 as K_2SO_4	78	$n/10000$	.....	100
7.	100 p.p.m. NO_3 as $NaNO_3$	201	.....	$n/5000$	401
8.	35 p.p.m. K as KCl	92	Neutral	Neutral	97
9.	100 p.p.m. NO_3 as $NaNO_3$ $n/620$ 63 p.p.m. K as KCl $n/620$	283	.....	$n/10000$	638
10.	100 p.p.m. NO_3 63 p.p.m. K } as KNO_3 $n/620$ 63 p.p.m. K as K_2HPO_4 $n/620$	295	.....	$n/20000$	250

These results show that six of the nine solutions became alkaline, two became acid, and one remained neutral. Growth and oxidation were less in the acid solutions than in those which became alkaline, although in the case of calcium chlorid the result was quite low. In the case of potassium sulfate and potassium chlorid a part of the depression may be due to the effect of the potassium, which usually fails to increase oxidation materially, but such is not the case with ammo-

nium sulfate. Neither is it probable that the sulfate radical is the depressing factor, since calcium sulfate compares favorably with calcium nitrate in its effect upon oxidation. The more favorable effect of no. 2 in Table X upon oxidation over nos. 3 and 4 is probably to be attributed to the presence of nitrate, which likewise appears to be responsible for a material increase in growth.

The greater oxidation accomplished by no. 9 over no. 10 is probably not to be attributed to the presence of Cl, but to the smaller amount of K present in no. 9.

On the whole it appears that while oxidation is affected to a certain extent by conditions of acidity or alkalinity arising in the culture medium, it is more materially affected by the specific action of the salts and their elements in the solution.

The direct effect of acid and alkaline conditions upon the activity of peroxidase was investigated by the following experiment in which alcoholic aloin was used as the indicator. A liquid showing strong peroxidase action was taken from a pan in which several hundred 7-day-old wheat seedlings were growing. Various amounts of $n/50$ HCl and $n/50$ NaOH were added to a set of tubes each containing 10^{cc} of the culture liquid and 1^{cc} of alcoholic aloin solution added at 3 P. M., January 13. The following table shows the amount of acid or alkali added in each tube, and gives the record of the colors observed at 11 A. M. the following day.

TABLE XI

Effect of acid and alkaline conditions upon the activity of peroxidase in the absence of plants.

No.	Solution	Color observed at end of 20 hours
1	10^{cc} culture liquid + 0.1^{cc} $n/50$ HCl	faint pink
2	" " " + 0.2 " "	" "
3	" " " + 0.5 " "	no change
4	" " " + 0.7 " "	" "
5	" " " + 1.0 " "	" "
6	" " " + 0.1 $n/50$ NaOH	red
7	" " " + 0.2 " "	wine-red
8	" " " + 0.5 " "	deep wine-red
9	" " " + 0.7 " "	" " "
10	" " " + 1.0 " "	" " "
11	" " " neutral to litmus solution	deep pink
12	" " " " " " "	" "

From these results it can only be concluded that a slightly alkaline medium is most favorable for this peroxidase reaction. It will be remembered that WOLLNY[37] found also that the oxidation processes in the soil were distinctly favored by slightly alkaline conditions.

The effect of putrefactive processes upon oxidation is another question which was briefly investigated. When a number of seedlings were placed without any support in water containing aloin (the entire root system, seed, and lower part of the plant, being thus submerged), it has been observed that the red color first produced subsequently disappeared. An experiment was accordingly planned to learn whether oxidation phenomena would be affected when the seeds were submerged and gave rise to products of putrefaction. Twelve cultures of wheat plants were prepared and allowed to grow four days in tap water. In one-third of the cultures the seedlings were adjusted in the notched corks so that only the root systems of the plants were submerged; in one-third of the cultures the seedlings were lowered so that the seeds also were submerged, and one-third had the seedlings entirely submerged. On the fourth day 100^{mg} of aloin were added to each culture jar, and they were examined twenty-four hours later with reference to the production of colors. It was found that the cultures planted with only the root systems submerged showed a very considerable amount of oxidation, but in those where the seeds or entire plants were submerged there was none of the red color produced by oxidation. In these cultures where no oxidation was shown, there were putrefactive processes at work, a fact which is taken to mean that the oxidation effects are not observed when putrefactive processes occur. Whether this inhibition of oxidation is caused by the products of putrefaction or by a perverted metabolism, since the plant must function under somewhat anaerobic conditions, remains undecided.

That the oxidizing power of the plant was not destroyed is shown by the fact that by raising the seeds out of the culture water and refilling the jars with fresh tap water containing aloin, the characteristic oxidation occurred.

The foregoing experiments raised a question as to the amount of

[37] Die Zersetzung der organischen Stoffe und die Humusbildung. Heidelberg. 1898.

oxidation which occurs in poorly drained soils, where putrefactive processes are known to exist. In investigating this question, two crops of wheat seedlings were grown in Arlington clay loam in paraffined wire pots, giving the pots different amounts of water.

Lot I of the pots was kept at the optimum water content of the soil. The soil in lot II was kept saturated with water from the start, and the soil in lot III was saturated after the wheat seedlings were up. The relative green weight of the first crop of wheat plants, which grew from February 18 to March 14, was: I, 100; II, 111; III, 104. The relative weight of the second crop, grown from March 17 to April 8, was: I, 100; II, 67; III, 116. Extracts of these soils were then made and wheat plants were grown eleven days in the various extracts. At the end of that time the growth and oxidizing power of the plants in the different solutions were determined with the result shown in Table XII.

TABLE XII

Growth and oxidation in extracts of soils of varying moisture content. Growth expressed in terms of relative transpiration.

No.	Soil treatments	Relative growth	Relative oxidation
1	Soil kept at optimum	100	100
2	Soil kept at saturation	78	69
3	Soil saturated after plants were up	151	11

These results show that the effects of the poor drainage conditions appear to be much more marked upon oxidation than upon growth. The soil, which was kept at optimum and only saturated after the plants had started, seemed to remain favorable to growth in the pots and in the extracts, but its extract was plainly not favorable to oxidation. In regard to the increase of growth, it should be remembered that this lot of soil was alternately very wet and dry during the course of the experiment.

EFFECT OF TOXIC COMPOUNDS UPON OXIDATION

Aside from the foregoing experiments, in which there were used extracts of soils which displayed toxic qualities toward plants, a few investigations were made upon the action of organic compounds whose toxic properties had been previously determined.

The organic compounds employed for this purpose were vanillin,

cumarin, and santonin. The compounds were dissolved in distilled water and the resulting solutions used as cultures, taking care that the concentrations chosen were not so great as to be fatal to wheat plants within the duration of the experiment. Vanillin was used at the rate of 100 parts per million, cumarin 10 parts per million, and santonin in a saturated solution, which was nearly 100 parts per million. The growth of the plants, as measured by transpiration and stated in figures, taking the growth of similar plants in distilled water as 100 in each case, was: vanillin, 63; cumarin, 81; santonin, 75. After the plants had grown in their respective solutions for 12 to 14 days, 100^{mg} of aloin were added to each and the results noted on the following day. The results agreed in showing no color indications of oxidation in any of the cultures where the toxic compounds were present, although the roots growing in the control cultures in distilled water showed by the red color produced that a material amount of oxidation had been accomplished.

That the mere presence of organic materials did not inhibit the oxidation was shown by an experiment employing a solution of leucine which was slightly beneficial to the growth of wheat seedlings in solution cultures. Solutions of leucine containing 50 to 100 parts per million, producing an increase in growth over distilled water of 54 and 98 per cent. respectively, were very favorable to oxidation and produced a much deeper aloin red than the cultures in distilled water.

It can only be concluded, therefore, that the toxic organic compounds studied were deleterious to oxidation because of their toxic properties, and it appears that they were even more deleterious to oxidation than to plant growth.

The oxidizing action of the plants upon toxic organic substances is a phenomenon which has been pointed out by the authors in a previous paper[38] and will be referred to again later. The experiments presented in that paper also showed that the addition of sodium nitrate and calcium carbonate to solutions of toxic organic compounds went far toward decreasing their harmful effects, and in some cases overcame them entirely. That the organic salts and the physiological activities of the plants working together had accomplished the destruction of toxic substances, was shown by both plant growth and

[38] Jour. Amer. Chem. Soc. **30**:85. 1908.

chemical tests. It now appears that while this destructive action of the plant upon the toxic body is going on, the oxidizing power in the presence of an excess, as it were, of toxic body is greatly reduced, and may even be entirely inhibited. The conclusion drawn from those experiments was that the plant roots are able to oxidize a certain amount of deleterious organic material, and that the presence of salts which favor oxidation increases the ameliorating action of the plant.

This question was studied a little further by an experiment in which the oxidation in solutions of toxic material was observed. A solution of cumarin containing 10 parts per million, with and without the addition of fertilizer ingredients, was used as a medium for plant growth and subsequently examined for powers of oxidation. Sodium nitrate was added to one portion of the cultures at the rate of 50 parts of NO_3 per million, and calcium carbonate at the rate of 2000 parts per million was added to another portion of the cultures. Wheat plants were installed in the cultures October 7 and grew until October 17. The oxidation was estimated by means of aloin. Table XIII gives the effect of this treatment upon growth and upon the oxidation in the toxic solutions and in control solutions to which no cumarin had been added. In each case growth and oxidation of the plants in distilled water are taken as 100.

TABLE XIII

Effect of sodium nitrate and calcium carbonate on growth and oxidation in solutions of cumarin. Growth expressed in terms of relative transpiration.

No.	Solutions	Relative growth	Relative oxidation
1	Distilled water	100	100
2	" " $+NaNO_3$	196	250
3	" " $+CaCO_3$	170	166
4	Cumarin 10 p.p.m.	81	31
5	" " $+NaNO_3$	159	139
6	" " $+CaCO_3$	110	131

The results of this experiment show, in harmony with the previous ones, that the addition of these fertilizer ingredients overcame to a large extent the deleterious effect of the cumarin upon growth, each one making the cumarin solution to which it was added a better medium for growth than distilled water. An inspection of the figures

expressing the relative oxidation shows, however, that the addition of these salts produced relatively greater increases in oxidation than in growth. When sodium nitrate was added to cumarin the resulting growth was twice as great as where only cumarin was present; the oxidizing power, however, was increased over fourfold. In comparison with this effect, it will be noted that the addition of sodium nitrate to distilled water likewise increased the growth twofold and increased the oxidizing powers two and a half times. It seems quite evident, therefore, that the ameliorating powers observed under the conditions of the experiment are to be referred to the increased oxidizing powers which are thereby brought about, and the consequent diminution in amount and activity of the toxic material.

It may be noted that LE RENARD[39] found that nitrates had a greater antitoxic value than other radicals when Penicillium was grown in the presence of copper.

SUMMARY

1. Roots of growing plants exhibit an extracellular oxidizing power which may be demonstrated by the use of suitable chromogens in nutrient solutions or soil extracts.

2. The oxidizing power appears to be most energetic in the region of the root where root hairs are found, and to decrease gradually in activity as that portion of the root becomes older.

3. The oxidizing power of plants grown in extracts of productive soils is greater than that of plants grown in extracts of unproductive soils.

4. Treating the soil extracts with an absorbing agent is usually beneficial to oxidation.

5. The distillate of a poor soil extract which contains volatile toxic compounds was less favorable to oxidation than the residue remaining from distillation.

6. The presence of toxic organic substances in solution was extremely deleterious to the oxidizing power of the plants. The oxidizing power of the plants, especially in the presence of nitrates, was able to alleviate the toxicity of such solutions.

[39] Essai sur la valeur antitoxique de l'aliment complet et incomplet. Paris. 1907.

7. The process of oxidation is usually accelerated by the addition of sodium nitrate to an aqueous soil extract. The addition of other fertilizer salts also influences oxidation.

8. The process of oxidation by roots is largely, if not entirely, due to the activity of a peroxidase produced by the roots. This oxidizing enzyme is most active in neutral or slightly alkaline solutions. The activity of the enzyme may be inhibited by the presence of acid and also by the conditions in solutions where anaerobic processes occur.

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BOG TOXINS AND THEIR EFFECT UPON SOILS[1]

ALFRED DACHNOWSKI

(WITH TWO FIGURES)

In the north and in the middle west, notably in Indiana and Illinois, and to some extent in Ohio and the adjoining states, there are extensive swamp areas of vast importance to the state, some of which are called "unproductive," and generally are not cultivated. The statement is made, and there is certainly much truth in it, that these swamp lands should be naturally very rich in constituents needed for plant food. Many of these places represent deep basins of accumulated plant débris, and the drainage from hills further enriches the accumulation in the swamps. They are noted for their dense and luxuriant surface vegetation. Examples of swamp and bog areas in this vicinity show clearly that bog water contains apparently all of the constituents required for the nutrition, growth, and reproduction of a large variety of grasses, shrubs, and trees. However, from an agricultural point of view these muck and swamp lands seldom have given satisfaction, even after drainage or addition of fertilizers. Ample proof of this is seen in the reports of the various experiment stations (**7, 8**). Thus far the remedies proposed, as a laboratory expedient, emphasize the fact that although some principles of soil fertility seem well established, and can be applied with definite results, there are yet many complex problems, the solution of which would materially enhance the economic importance of peat and swamp soils.

To the writer it has seemed probable for some time that work upon the chemistry and upon the physiological properties of peat and humus compounds must result in data valuable alike to the agriculturist, the forester, and the ecologist. Through an investigation on the cause of xerophily in bogs (**5**), there was gained supplementary evidence, of a more direct and positive sort, that the inhibiting factors of a bog are in part the presence in the soil water of injurious toxic sub-

[1] Contribution from the Botanical Laboratory of Ohio State University, XLIII. This paper was in part read before Section G of the A.A.A.S., at the Baltimore meeting, December 29, 1908.

stances. In that publication experimental data were given to show that the toxicity of bog water and of the bog-soil substratum can be corrected by various methods, and that the plants grown in solutions thus treated show not only accelerated growth and an increase in transpiration, but also an increase in the green and dry weight of organic matter.

It is not known, as yet, whether the toxic action of bog water and bog soil is determined by the action of one constituent or by the combined action of several. The experiments so far completed have given no definite evidence that the toxins are merely specific excretions from the roots and rhizomes of bog plants. Preliminary tests, not here detailed, which were carried on in the winter of 1907, indicate that the toxicity may be due to a certain unstable body, of the nature of organic compounds excreted from the roots in the absence of O_2 and in heavy clay soils not adequately aerated. It is probably a product of imperfect oxidation and decomposition of proteins and other related substances, and it is possible that in respiration bog plants differ from other plants. Since then an excellent account has appeared by STOKLASA (**12**), in which similar results on the excretions by roots are announced. Largely, however, the toxicity of bog water seems to be due to another cause. During the changes which the accumulated plant material undergoes in the process of peat-making, there are alterations and reductions leading to gaseous and colloidal products but little known. The relative amount of these varies with the seasons, and no doubt also with the locality, but primarily it depends upon the stage in the progress of decomposition. CH_4 and H_2S, though produced in small quantities, have been found to constitute the principal gaseous products. They are especially noticeable when well-corked jars of bog water remain standing for some time. Studies on the character of the colloidal products are still in progress. The injurious products of a microflora accumulating in definite layers of the soil are, perhaps, an additional factor to be considered. Indeed, it is a serious difficulty in physiological ecology that a process must be assigned to a single category or broken up into a number of what often prove to be arbitrary categories, in order to arrive at results in any way intelligible. Too often is there a tendency to lay undue emphasis upon "average"

conditions; and the more detailed responses which are due to localized influences are thus neglected. On the other hand, even though we decide experimentally which of the physical and chemical variables involved is of greater influence at a given stage, it still remains to determine how the *ensemble* of factors acts in the process which accompanies each physiographic change and serves as the functioning basis for morphological differences.

In connection with the experiments on the presence of injurious substances in bog water and bog soils, and their effect upon agricultural plants, the question arose whether the toxins which are harmful to plants in water cultures are injurious also to plants growing in soil containing them. This question has an added interest just now, because facts like those cited above give indications that the sterility of unproductive and "exhausted" agricultural soils may partly be caused by some toxic substance of a similar physiological and chemical origin. Different workers have observed that the growth of plants often gives rise to unfavorable conditions. The data obtained from various lines of experiments all go to prove that "exhaustion" cannot always be attributed to the removal of plant-nutrients from the soil by previous crops or by previous plant societies (**10**). To attempt a review of the literature on this problem would be out of place in the present paper. Suffice it to say that the results thus far obtained point strongly to the view that decreased physiological activity of plants lies rather in the toxic condition of the soil. The experimental proof is still regarded by many as furnishing negative evidence upon the problem (**6, 9**), and hence a spirit of controversy prevails in most of the writings upon this subject. However, it can no longer be questioned that the solution of this inquiry is of great importance to agriculture. It promises to throw new light upon many interrelations of soil and plants, and appears to afford a satisfactory explanation of some of the problems connected with the association and succession of plants, which on every other criterion would largely remain an enigma.

For the purpose of determining whether the toxins of bog water are harmful also to plants growing in soils containing the injurious substances, it was decided to employ first of all a soil medium as nearly non-nutrient as possible. Quartz is one of the chief and most

nearly insoluble constituents of soil. It has been shown (2) that quartz is of minor importance in the adsorption and retention of hydroxids and various neutral salts; a knowledge of its action for bog water seemed, therefore, of fundamental importance. The quartz used was obtained from the Ceramics Department of the university. To free it from possible impurity it was subjected to a thorough washing. The air-dry quartz sand was first sifted through a sieve having meshes of 1^{mm}. Portions of about 250^{gm} of the sifted material were each placed in a large porcelain dish containing distilled water acidulated with HCl. It was usually the practice to boil the material for twenty minutes. After boiling, the supernatant liquid was decanted and fresh distilled water was added. A similar washing was carried out in *aqua regia* and later again in dilute KOH. The quartz was then washed repeatedly in boiling distilled water and finally dried at 100° C until ready for use.

The bog water used in these experiments was collected from the same central station on the bog island as described in the earlier paper. The solution is relatively clear, the suspended particles imparting to it a slight tinge of olive green to brown. It is very little acid to phenolphthalein, but alkaline to methyl orange.

Since no experiments had been made thus far to ascertain how much of the toxic property of bog water is removed by a given quantity of an adsorbing agent, series of ten cultures were prepared for this purpose. Seven of the cultures consisted each of 400^{cc} of bog water, to which was added sterilized quartz in quantities equivalent to the following volumes: 25, 50, 75, 100, 125, 150, and 200^{cc} respectively; that is, the quantities were chosen in volumes equal to a definite fraction of the volume of bog water used. The bog water and the quartz sand were shaken together in glass-stoppered bottles, and left standing for several days. When ready for use the liquid was decanted and placed in half-liter Mason jars, covered with black paper. Three control cultures were added, consisting respectively of untreated bog water, boiled bog water, and distilled water. The wheat seedlings used for these cultures were germinated in sawdust until 4 to 5^{cm} high. In later experiments the seedlings were germinated in quartz sand. They were then carefully washed in distilled water and transplanted to the water cultures. Never less than six

seedlings were used in any experiment. It should be observed also that the seedlings were selected individuals out of a large number of plants. The corks used were previously sterilized and paraffined. Growth was measured by transpiration and the green and dry weight of plants. The cultures stood side by side in the university greenhouse in diffused light. The weekly atmometer readings varied between 176 and 186^{cc}. Below are given toxicity figures for bog water collected at two periods. Column I gives data for bog water collected September 12, 1908, nearly at the end of one of the most severe droughts that have been experienced in Ohio; the bog water for column II was brought to the laboratory October 16, soon after the first rains. The evidence derived from similar experiments with bog water collected at intervals of one month during the year is omitted, showing, as it does, considerable repetition. It should be noted, however, that the variation in the range of results for the seasons is considerable.

TABLE I

ADSORPTION OF BOG TOXINS BY QUARTZ SAND

SOLUTION	CORRESPONDING PLACE ON CURVE	TOTAL TRANSPIRATION FOR 15 DAYS, IN GRAMS	
		I. Sept. 12	II. Oct. 16
1. Distilled water 400^{cc}	A	7.50	7.50
2. Bog water 400^{cc}, untreated	B	10.26	14.90
3. Bog water 400^{cc}, boiled	B′	.....	54.22
4. Bog water 400^{cc} + 25^{cc} SiO_2	C	22.70	25.50
5. Bog water 400^{cc} + 50^{cc} SiO_2	D	.....	18.17
6. Bog water 400^{cc} + 75^{cc} SiO_2	E	39.28	13.83
7. Bog water 400^{cc} + 100^{cc} SiO_2	F	.....	13.56
8. Bog water 400^{cc} + 125^{cc} SiO_2	G	48.73	.....
9. Bog water 400^{cc} + 150^{cc} SiO_2	H	.....	12.87
10. Bog water 400^{cc} + 200^{cc} SiO_2	K	18.60	12.55

The results for these two dates have been plotted in *fig. 1*. The growth-rate in terms of transpiration is indicated on the axis of ordinates, and the progressive addition of quartz to bog water is shown on the axis of abscissas.

Before taking up the facts brought out in this series of experiments, another part of the investigation must be mentioned here. The foregoing observations suggested the query whether results obtained with soils of varying quality, fineness, and adsorbing surface would

show that the toxic strengths of the same bog-water solution have approximately the same relation to each other irrespective of the nature of the filter used. It was intended to use types of soil ranging progressively through the weathering products from feldspars to kaolin. But the feldspars are highly alterable minerals, and the chemical products of feldspathic and granitic rock-decomposition

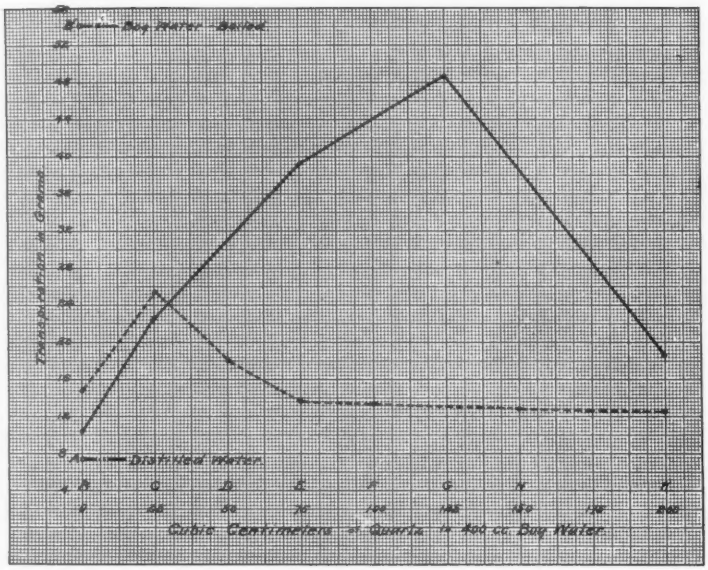

FIG. 1.—Diagram showing growth-rate of wheat seedlings in treated bog water. The ordinates represent transpiration in grams; the abscissas show the progressive addition of quartz to bog water. Unbroken lines for bog water collected September 12, 1908. Broken lines for bog water of October 16, 1908. Broken line single dotted for boiled bog water; broken line double dotted for distilled water.

are extremely varied (4). In the residues, however, which remain after leaching, free silica as quartz, and a number of rather indefinite substances known as clays, are the most abundant. In the present case the efficiency of the following substances, characteristic of the final residue of soil-forming rocks, and their allied substances, was tested: SiO_2 coarse; SiO_2 fine; kaolin; $CaCO_3$; SiC; and C in the form of air-dried humus. The materials were obtained through

the courtesy of Mr. C. H. KERR of the Carborundum Company, Niagara Falls, New York. They are among the most insoluble substances known, and of great purity, which makes them of special value in this investigation. The chemical analysis of these materials is as follows:

TABLE II

Material	Quartz	Kaolin	Carborundum
C	.....	.....	29.71
O	.....	.....	0.45
Si	.....	.....	69.40
SiO_2	99.56	45.35	.....
Al_2O_3	0.40	38.86	0.28
CaO	.....	0.35	0.15
Fe_2O_3	.....	0.38	.....
K_2O	.....	0.61	.....
MgO	.....	0.31	0.05
Na_2O	.....	0.31	.....
Ignition	0.14	13.80	.....

The physical composition of the materials employed was determined by microscopic examination. Mechanical analysis was made with the aid of a centrifuge and for the coarser components by means of their different rates of subsidence in water. The relative percentages by weight of the different component particles in each group is as follows:

TABLE III

Material	SiO_2 coarse	SiO_2 fine	Kaolin	SiC
Sand 0.5—0.25mm	100	....	....	....
Very fine sand 0.1—0.05mm	...	6.5	2.4	79.4
Silt 0.05—0.005mm	...	80.8	61.8	16.6
Clay 0.005—0.0mm	...	12.9	35.7	4.5

To obtain the surface area of spherical particles it is only necessary to invert the value of the mean diameter of the particles for each group. This surface factor is then multiplied by the fractional amount of the quantity of the sample having particles of these mean diameters. A mathematical calculation of the surface area of quartz flour, carborundum, or other crystalline bodies with irregular

surfaces, however, is not so readily obtained. It may be that adsorption of toxins and adsorption of vapors and gases are subject to the same conditions (**11**). Perhaps by taking measurements upon the rate of retention of a silver salt, one may secure an indirect method for the calculation of the surface of these bodies. A curve showing how the adsorption data are related to the surface presented by the grains of the different soils used, though of interest, is not a question at issue in this discussion, but it is hoped to continue this problem further, and in a more quantitative manner.

The precaution was taken to allow contact between the solution and the solid bodies for thirty minutes only, in order to reduce to a minimum the low solubility of the materials (**3**) and the possible action of the solution upon the solids. The amounts used in each case, and the effect of these insoluble substances on the toxic action of bog water collected January 30, 1909, are given below in Table IV. The transpiration data cover a period of fifteen days and are for six wheat plants in each solution.

TABLE IV

ADSORPTION OF BOG TOXINS BY INSOLUBLE SUBSTANCES

SOLUTION	TRANSPIRATION IN GRAMS; SIX WHEAT SEEDLINGS IN EACH SOLUTION			
	5th day	10th day	15th day	Total
1. Bog water 400cc, untreated.....	4.30	8.55	3.65	16.50
2. Bog water 400cc, filtered.....	4.90	10.35	6.75	22.00
3. Bog water 400cc + 15cc SiO_2 (coarse).	5.25	10.00	7.35	22.60
4. Bog water 400cc + 25cc SiO_2 (coarse).	7.20	12.85	9.80	29.85
5. Bog water 400cc + 50cc SiO_2 (coarse).	7.00	13.55	10.65	31.20
6. Bog water 400cc + 15cc SiO_2 (fine)...	8.60	19.10	13.50	41.20
7. Bog water 400cc + 25cc SiO_2 (fine)....	6.70	19.00	16.60	42.30
8. Bog water 400cc + 50cc SiO_2 (fine)....	7.68	16.40	13.10	37.18
9. Bog water 400cc + 15cc Kaolin.....	9.10	20.00	16.25	45.35
10. Bog water 400cc + 25cc Kaolin.....	9.65	19.32	21.58	50.55
11. Bog water 400cc + 50cc Kaolin.....	9.95	20.10	23.40	53.45
12. Bog water 400cc + 150cc Kaolin.....	11.98	22.20	16.07	50.25
13. Bog water 400cc + 15cc $CaCo_3$.....	10.90	20.50	24.70	56.10
14. Bog water 400cc + 25cc $CaCo_3$.....	10.48	20.70	26.50	57.68
15. Bog water 400cc + 50cc $CaCo_3$.....	10.07	19.17	23.35	52.52
16. Bog water 400cc + 15cc SiC (fine).....	11.23	21.25	25.60	58.08
17. Bog water 400cc + 25cc SiC (fine).....	10.90	17.50*	21.20*	48.60*
18. Bog water 400cc + 50cc SiC (fine).....	11.25	23.00	29.10	63.60
19. Bog water 400cc + 15cc C. (humus)...	11.00	25.15	40.65	76.80
20. Bog water 400cc + 25cc C. (humus)...	8.30	16.70	41.90	66.90
21. Bog water 400cc + 150cc C. (humus)...	8.55	22.15	24.80	55.50

* 5 plants in culture.

Several facts seem to be clearly brought out in the above data. A comparison of the toxicity figures of bog water collected at intervals during the year indicates that the amount of toxic substances in solution differs very appreciably within the year. In all cases the physiological studies show that the adsorbing substances actually remove definite quantities of bog toxins. In contrasting the efficiency of the various amounts of adsorbents used, the important facts at the outset are these. Different physiological phases result from the progressive increase of an adsorbing substance. The bog-water solution, fatal in its effect at some seasons, gives an increase in growth-rate when adsorption removes a sufficient amount of the toxic ingredient. The effect is virtually one of dilution. Doubling of the amount of the adsorbent brings the growth-rate into a physiological phase marked by a greater functional activity. Further addition and its consequent further dilution in toxicity carries with it a corresponding intensification in growth-rate. The appearance of the plants, especially in the development of the root system, follows the transpiration figures very closely (see **5**, *figs. 1, 4–5*, p. 135). Stimulation and tolerance rise to a maximum. But with successively larger amounts of adsorbent, the optimum rate of transpiration can be neither increased nor maintained. It falls off, regularly and rapidly at first, subsequently less rapidly, until the effect of the solution is practically that of distilled water (**13**). Greater dilution and consequent increase in rate of transpiration does not express therefore the whole truth. Other and less injurious substances are also adsorbed; and the rate of transpiration is seen to be the product of a coordination of factors (**1**). In bog water with very slight toxicity, the course of the experiment shows that the maximum acceleration phase deviates very sensibly toward the growth-rate approximated in the control i. e., the untreated bog water.

On account of the difference in size of particles, there naturally follows a corresponding difference in the amount of adsorption. Compared with kaolin, calcium carbonate, carborundum, and carbon (as lampblack or humus), the adsorptive power of quartz is relatively low. It will be seen that the optimum rate of transpiration of the tenth day, in solutions 3 to 8, Table IV, is soon succeeded by a minimum. This is due to the action of toxic substances still

present; for upon further addition of adsorbents the minimum at the end of the fifteenth day is succeeded by a higher rate of transpiration. Filters of finer grain are more beneficial, while the adsorptive power of humus is very much higher than that of any of the crystalline substances used. The optimum and maximum rates of transpiration occur on the fifteenth and twentieth day and lie near together. Reference to the total amount transpired shows that the adsorption of carborundum and humus is about three to four times greater than that of quartz. The transpiration data serve excellently as a basis for assigning a limit to the magnitude of the toxic effect, and as an expression of the amount of the unknown body adsorbed both in terms of the total adsorption, and as a percentage of the surface factor of grains. The results with $CaCO_3$ also show that the plants are not affected by conditions of acidity or alkalinity, and that growth seems to be more materially affected by the specific action of the organic toxins present. Whatever the nature of the filter used, that the increased tolerance of wheat seedlings to bog water is actually due to the adsorptive power of the filters is sustained by the fact that the decrease of the poisonous effects of bog water is apparently a function of surface of particles and is approximately proportionate to the quantity of the solid body used. The solution, decidedly toxic without the solid, becomes capable of supporting a more than normal growth.

The outcome of these preliminary tests is, therefore, that the conditions giving rise to decreased physiological activity, to xerophily, and to zonation of bog plants are not found in the depletion or increase of mineral nutrients in bog water, nor in a low soil temperature, but lie rather in the toxicity of the soil substratum, i. e., in the production of unfavorable soil conditions brought about by the plants themselves.

However, experiments by the water-culture method may not always be serviceable as a safe basis for argument concerning soil conditions. A number of life relations of the plant in a water culture become changed when in the soil. In what particular manner the toxic bodies are held by the adsorptive force of the filters is not clear if judged by physical or chemical analysis. The marked retention of the toxins of bog water observed may be due to causes other than a direct condensation on the surface. No experiments were made to

show conclusively that the retention is not due to chemical fixation or substitution. The amount of solution thrown out of the quartz by the centrifugal machine was too small to be tested. A priori, it would seem that the filter used should be markedly more toxic now than the solution, when tested by physiological criteria. The presence of the adsorbed bodies in the solid should not only reduce its effectiveness when repeatedly used for improving bog water, but should replace normal growth by an abnormal retardation judged from the growth-rate made in a similar check soil culture.

To obtain evidence on this point, and to contrast the efficiency of the various constituents of agricultural soils for adsorption, a series of experiments was made with quartz, river sand, field clay, and humus soil. The air-dry soils were sifted through a sieve with meshes of 1^{mm}. Portions of 400^{cc} of the sifted soils were each placed in glass-stoppered jars containing 1200^{cc} of bog water. The glassware employed in all of the experiments cited was treated with a solution of potassium dichromate and sulfuric acid, and repeatedly rinsed in distilled water previous to use. The mixtures of bog water and soil were left standing in the dark room for three days. To insure thorough contact between the bog water and the soil, the solution was occasionally shaken. When ready for use the liquid was filtered off, and portions of 400^{cc} of the liquid from each soil type were used as water-culture experiments in the manner described above. For the investigation on the relative fertility of the soils used as filters, earthenware pots were used. The pots were new and each of about 300^{cc} capacity (8^{cm} in diameter, 8.5^{cm} deep). They were thoroughly cleaned and dried in an oven at 110° C, and later immersed in heated paraffin. To each paraffined pot was added 200^{cc} of the contaminated soil well pressed into the bottom and sides of the pots. It was recognized that difficulties of obtaining good contact between the soil and the walls of the pot would be probable. In the air space along the walls usually by far the greater proportion of plant roots are developed, and the wire-basket method as recommended by the Bureau of Soils of the U. S. Department of Agriculture (Bull. 23) is, therefore, more desirable. But the form of retainer here described was found to be wholly satisfactory. In no case were evidences found of roots growing more freely at the sides of the pot than in the center. The experi-

ments were repeated later by the wire-basket method with the same results. Six wheat seedlings were transplanted in a row in the soil of each pot. In identically the same manner a series of duplicate cultures with the untreated soils was prepared to serve for comparison with the behavior of wheat seedlings in the contaminated soils. The filled pots were then weighed and placed in the greenhouse where they stood side by side. Direct sunlight was avoided by cloth screens. Only one of the experiments need be given, and Table V gives a summary of the results obtained with bog water collected September 12, 1908. The percentage increase is calculated upon the basis of the quantities marked zero (0), considering them as unity for the respective series. The photograph (*fig. 2*), which I owe to the aid of Professor J. H. SCHAFFNER, shows these plants at the end of the experiment.

TABLE V

ADSORPTION OF BOG TOXINS BY SOILS

SOLUTION	AVERAGE LENGTH IN CM.		PERCENTAGE INCREASE		
	Tops	Roots	Transpiration	Green weight	Dry weight
1. Bog water untreated.....	15.8	5.3	0	0	0
2. Bog water quartz-filtered...	20.8	42.	338.	134.	56.
5. Bog water clay-filtered.....	19.9	11.4	154.	68.	11.
8. Bog water humus-filtered..	30.5	15.6	805.	220.	84.
3. Contaminated quartz soil..	22.	12.3	....	0	0
6. Contaminated clay soil....	22.2	6.6	...	0	0
9. Contaminated humus soil...	21.9	6.2	...	0	0
4. Control quartz soil.....	24.7	9.6	...	8.	20.
7. Control clay soil.....	26.	11.7	...	23.	5.
10. Control humus soil.....	30.7	13.5	...	86.	55.

Again it is evident that the addition of solids has increased the tolerance of the seedlings to bog water. The improvement was marked during the entire period of experimentation. The presence of the toxic bodies in small amounts exerted a noticeable stimulating effect, while the plants in the control bog water gave every indication that they would be unable to survive an exposure of a normal growing period. The last-mentioned point has been repeatedly tested also in field-work. It seems as if the roots, and especially the more minute roothairs, of the plants in the untreated bog water served as adsorbing surfaces. The roots are brownish in color and jelly-like in consist-

ency; deposited upon their surfaces are found numerous colored bodies, as the result of the oxidizing action of the roots. The nature of these bodies is still under investigation. A general decay of the growing tips is noticeable, showing that the oxidizing action of the plants upon the toxic substances went far toward decreasing their harmful effect, but could not entirely overcome them. The effectiveness of adding the insoluble solids proves, therefore, very conclusively

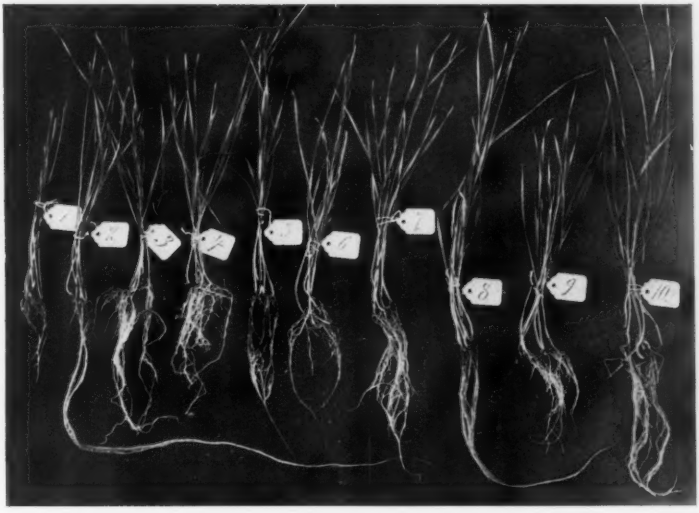

FIG. 2.—Growth of wheat plants in various cultures of bog water. Numbers as in Table V.

that the source of the harmful condition must logically be looked for in the solution and not in the condition of the plants themselves. The difference in the tops as well as in the roots of the plants from the various cultures is very striking. The stimulation effect is less marked in the solutions filtered through clay and humus, because of the greater adsorptive power of these substances; yet the increase in the green and dry weight of plants is relatively twice that in the untreated bog water while transpiration has increased almost tenfold. The introduced materials have had their adsorptive action, but it is evident

also that chemical reaction enters in the case of the common types of garden soil.

We come finally to a consideration of the effects of bog toxins upon soils. It is to be noted that the poisonous action of bog toxins is more harmful when the plants are immersed in the solution, than when grown in the contaminated soil cultures. That the poisonous matter injurious to plant growth is present in the soils used as filters is seen upon comparison with the controls. Manifestly, the theory of lack of O_2 in bog water or in bog soils as the cause of xerophily is not satisfactory to account for the results, because water cultures usually have less O_2 than any soil medium. The transpiration data for boiled bog water (Table I, page 393) are further evidence in this direction. The inadequacy of the theory of low substratum temperature is, for this locality, equally obvious. That the action cannot be attributed to large amounts of dissolved substances has been shown in the determination of the osmotic pressure of bog water in the author's earlier paper (*l. c.* **5**). The garden soils contain a much larger amount of nutrient ingredients than bog water, and hence the presence of those salts should tend to increase the growth-rate. No such increase in activity occurred. The length of time during which the wheat plants were allowed to grow is palpably insufficient to "exhaust" or contaminate the soils. The retardation seen in the contaminated soils is lacking the corresponding normal average in dry weight of plants to an amount of 18 per cent., 3 per cent., and 36 per cent. for quartz, clay, and humus respectively. From the results it may be concluded that the adsorption and retention capacity of soil for toxins is generally higher the greater its content of humus. It was shown elsewhere that a bog-water solution well aerated, or upon long standing with exposure to air, lost its injurious properties. When plants are grown in this oxidized solution it is found that the solution becomes decidedly beneficial to plant growth. These results are also obtained with the contaminated soils. When first used they exert a distinctly injurious effect. If the amount of water transpired by the plants is replaced by bog water, the soils become more toxic. Decrease in toxicity always follows aeration of the soil and drainage; and since the physical conditions mainly determine the amount of oxidation, these are of greater consequence in restoring the fertility to the soil.

SUMMARY

The available information of the study here reported may be summarized as follows:

1. Many swamp and muck soils exhibit a sterility which cannot be remedied by drainage or by the addition of fertilizers.

2. The sterility appears to be most marked where investigations on the physiological properties of bog water and bog soils indicate a greater amount and activity of bog toxins.

3. The production of bogs toxins is due to a number of physical and chemical factors. One can only conclude that the chemical constitution of bog water and bog soils at a given moment conditions toxicity; and that the excretion from roots and rhizomes of plants is one of the variables of the conditioning factors.

4. In untreated bog water there are found deposited upon the roots of wheat plants numerous colored bodies as the result of the oxidizing action of roots. The general decay of the root-tips indicates that the oxidizing activity is insufficient to decrease the harmful effects of bog toxins.

5. It is possible that ecesis, association, and succession of plants depends primarily upon respiration, and that in respiration bog plants differ from other plants.

6. Treating bog water with an insoluble adsorbing agent is invariably beneficial.

7. Different physiological phases result from the progressive addition of an adsorbing substance. With coarser-grained materials the low optimum rate of transpiration is soon succeeded by a minimum which is due to the action of toxic substances still present.

8. Finer-grained insoluble bodies are more beneficial. The response to toxic bodies when present in small amounts leads to acceleration of growth. The period of growth is more prolonged, and the optimum and maximum rate of transpiration lie near together.

9. The adsorptive action of carborundum and humus is about four times greater than that of quartz; the capacity of soils for retaining toxins is therefore higher the greater the content of humus.

10. The decrease of the poisonous effect of bog water is probably a function of the surface of the particles; it is relatively proportionate to the quantity of the solid body used.

11. In agricultural soils used as adsorbents the presence of the adsorbed unknown toxins replaces normal growth by an abnormal retardation. Fertility is restored through aeration, that is, after time enough has elapsed for the oxidation of the injurious bodies.

12. The contaminated condition of agricultural soils and the consequent decreased physiological activity of the plants grown in them still further indicates that xerophily cannot be due to acidity, lack of oxygen, low temperature, etc., of the soil substratum; that is, the factors heretofore cited are only in part the cause of xerophily.

In view of the evidence presented above, the writer believes that these facts in the action of bog water upon soils justify the conclusion that there are present in bog water and in bog soils injurious substances which are, at least in part, the cause of xerophily in plants, and of decreased fertility in bog soils.

Grateful acknowledgment is made to Professors McCALL and VIVIAN, of the Agricultural College of the university, for the facilities of their laboratories, which were freely and courteously placed at the writer's disposal.

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BRIEFER ARTICLES

PARTHENOGENESIS IN *PINUS PINASTER*

(WITH SEVEN FIGURES)

In the course of an investigation on the life-history and development of the embryo of the cluster pine, *Pinus pinaster* Soland., it has occasionally been noticed that in some ovules containing proembryos in all stages of development either no trace of pollen tubes could be seen in the nucellar cap, or the tubes only extended through a part of the nucellus and no nuclei could be found in them. This strongly suggested the occurrence of parthenogenesis, but might have been due to imperfect preparations. Therefore, in 1908, collections were made about every twelve hours during the time when the archegonia mature, in the hope of obtaining more satisfactory evidence.

Great care was used in fixing and imbedding this material, and the following fixing agent has been found more satisfactory than any other, including chromacetosmic mixtures.

Picric acid, saturated solution in 50 per cent. alcohol, 100^{cc}; corrosive sublimate 5^{gm}; glacial acetic acid 5^{cc}. This fixing agent is mentioned by CHAMBERLAIN,[1] and I have to thank Mr. A. J. BALLANTINE for suggesting its use. Cedar-wood oil has been found much superior to xylol to precede the infiltration with paraffin, as mentioned by Miss FERGUSON (**'04**). The stains used have been Delafield's hematoxylin, much diluted and allowed to act for several hours, and Flemming's safranin gentian-violet orange-G combination. The first named shows nuclear details more sharply than the triple stain and is only equalled in this respect by Haidenhain's iron alum hematoxylin, which is more troublesome to use and in no way superior. In other respects the methods used have been those generally employed in cytological work.

The evidence obtained shows clearly that parthenogenesis occasionally occurs, and the most conclusive preparations are shown in *figs. 1* and *2*.

The points which seem to prove satisfactorily that the oosphere develops without fertilization taking place are as follows:

1. Although the oosphere nucleus has divided or begun to divide, the pollen tube has not yet reached the archegonium and still contains both the sperm nuclei (*figs. 1, 2*).

[1] CHAMBERLAIN, C. J., Oogenesis in *Pinus Laricio*. BOT. GAZETTE **27**:268–280. *pls. 4–6*. 1899.

2. A careful study of the other sections of the series has failed to show any other pollen tube which might have reached the archegonium from another direction.

3. The spindle of the

EXPLANATION OF FIGURES

All sections 6–8 μ thick; cut with the Cambridge rocking microtome; drawn with camera. In all: *n*, nucellus; *p*, prothallus; *t*, pollen tube; *a*, archegonium; *v*, receptive vacuole; ♀, oosphere nucleus; ♂, functional sperm nucleus; ♂₂, secondary sperm nucleus.

FIGS. 1 and 2.—Stages in the first division of a parthenogenetic proembryo; diagrammatic; reconstructed from several sections in each case. ×77.

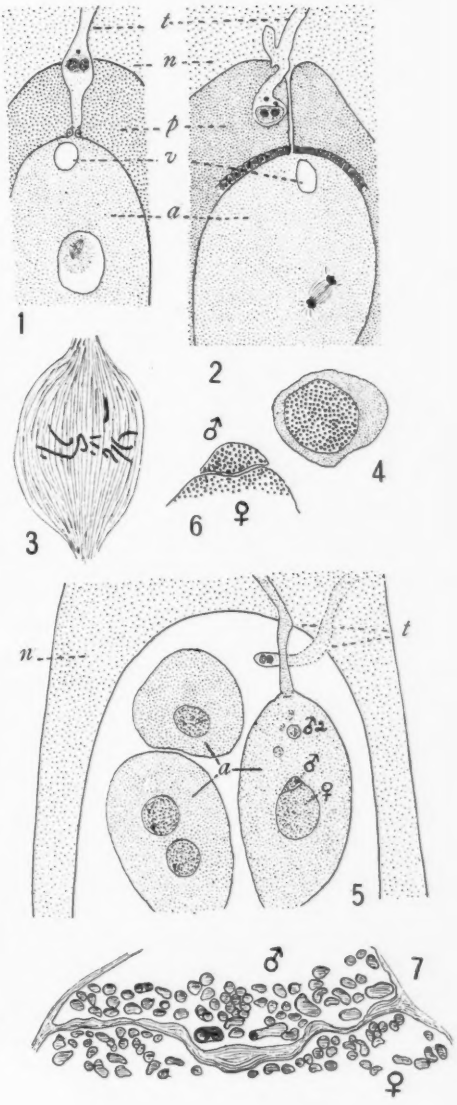
FIG. 3.—Spindle of *fig.* 1, showing the comparatively small amount of chromatin substance; drawn from two sections. (No attempt has been made to show all the chromosomes.) ×640.

FIG. 4.—One of the sperm nuclei of *fig.* 1, showing that these are quite normally organized. ×310.

FIG. 5.—Diagram showing normal fertilization in one of the archegonia. ×60.

FIG. 6.—Sperm nucleus and part of the oosphere nucleus of *fig.* 5. ×255.

FIG. 7.—Part of *fig.* 6. ×1240.



first division of the oosphere nucleus is parallel or oblique to the long axis of the ovule and is approximately in the center of the original nucleus. The normal spindle, on the other hand, is more or less transverse to the long axis of the ovule and lies quite at the top of the original oosphere nucleus. In both cases this spindle is entirely intranuclear, as shown by CHAMBERLAIN,[2] and some of the original achromatic nuclear material is not used up, but contracts considerably from the original nuclear membrane. (This contraction may be due to the action of fixing or other reagents, but as it is equally present in all preparations, whether or not any contraction has occurred elsewhere, I am inclined to think it normal.)

4. In normal fertilization a segregation of the chromosomes into two groups occurs both in the first and second divisions of the oospore nucleus, but no segregation can be seen here (*fig. 3*). The chromosomes are long and rather irregular in shape and are often cut into several pieces and distributed through as many sections. It has therefore been impossible to count them accurately, but the number in the normal sporophytic nucleus is certainly in the neighborhood of 24, and in the spindle of *fig. 3* it is as certainly less than that number.

5. In normal fertilization a good deal of disorganization of the apical part of the archegonium occurs, and the receptive vacuole is either broken or considerably displaced (*fig. 5*). In the archegonium from which *figs. 1* and *2* were drawn, no such disorganization has occurred, and the receptive vacuole occupies its normal position.

6. In normal fertilization the remains of the second sperm nucleus and the tube nucleus and the stalk cell can be distinguished for a time in the upper part of the archegonium, but no trace of these nuclei can be found in the archegonia of *figs. 1* and *2*.

As far as has been seen, the abortion of the ovule frequently occurs before the formation of a proembryo, but never after. A large number of preparations of the proembryos and embryo in all stages of development leaves no doubt on this point. Hence it appears that parthenogenetic embryos must develop as well as normal ones. It is impossible to say whether this development is only intraseminal, or whether seeds containing such embryos are able to germinate and produce normal plants.

Fig. 5 shows, for the sake of comparison, a case of normal fertilization, and the conjugating nuclei are shown in more detail in *figs. 6* and *7*. The preparation shows very clearly that the nuclear membranes are not in

[2] CHAMBERLAIN, C. J., Methods in plant histology. Second edition. The University of Chicago Press. 1905.

contact, but separated by a thin layer of cytoplasm, as mentioned by Miss FERGUSON.[3]

In *fig. 5* the second pollen tube is evidently on its way to the smallest of the three archegonia, and is taking its way laterally through the tissue of the prothallus instead of down the canal leading to the neck.

The third archegonium is apparently sunken in the tissues of the prothallus, but unfortunately the series is incomplete, and it may have only a very obliquely placed neck. For the same reason it is impossible to say whether this proembryo is really parthenogenetic, as it appears to be.—W. T. SAXTON, *South African College, Cape Town.*

CARNATION ALTERNARIOSE[4]

(WITH EIGHT FIGURES)

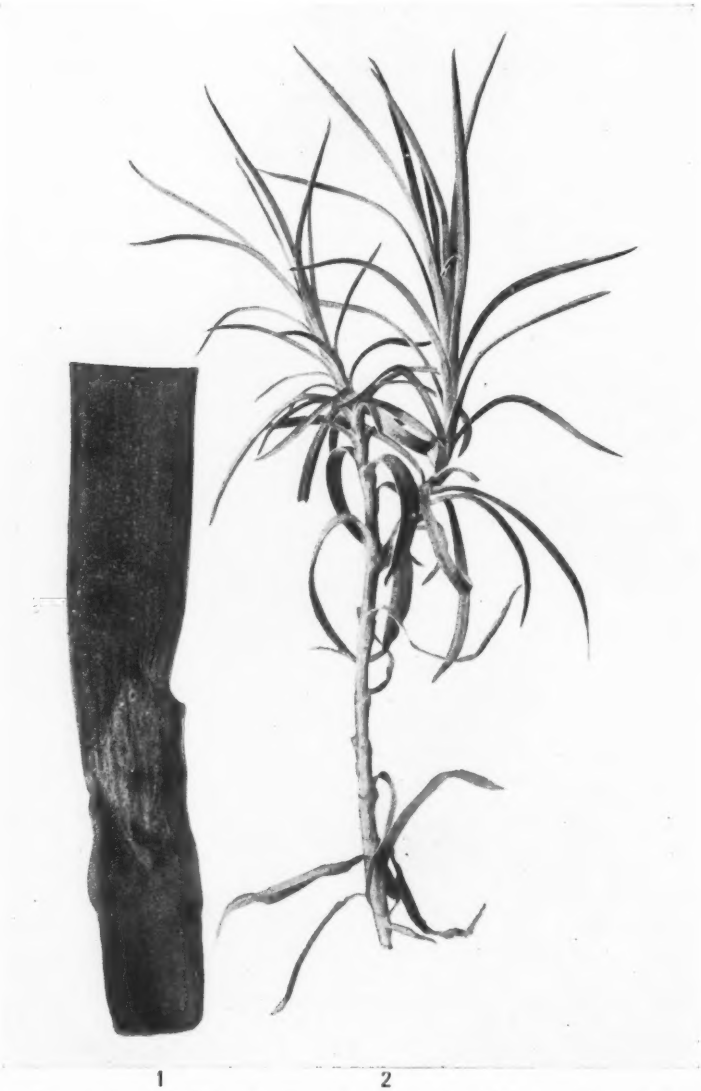
To a leaf-and-stem disease of the cultivated carnation, *Dianthus Caryophyllus* L., our attention was called by local florists as causing serious damage. The disease, upon examination, proved to be one hitherto undescribed and a laboratory study of it was undertaken.[5]

Symptoms.—The disease manifests itself as spots, mostly upon the leaves, sometimes upon the stems, especially at the nodes. These spots are strikingly characteristic, of ashen whiteness, with the center occupied by an often scanty, though sometimes profuse, black fungous growth. The diseased spot is dry, somewhat shrunken, thinner than healthy portions of the leaf, approximately circular, though often somewhat elongated in the direction of the longitudinal axis of the leaf (*fig. 1*). When occurring at the node, the disease usually involves the bases of both of the leaves, as well as the stem between them (*fig. 2*). As these nodal spots age, the disease penetrates through the stem, killing its tissue, which shrinks some-

[3] FERGUSON, M. C., Contributions to the knowledge of the life-history of Pinus, with special reference to sporogenesis, the development of the gametophytes and fertilization. Proc. Washington Acad. Sci. **6**:1–202. *pls. 1–24.* 1904.

[4] This termination was suggested by the authors in Annales Mycologici **7**:49. 1909, with the following explanation: "We believe that much will be gained both in clearness and brevity by designating diseases in plants by the uniform termination 'ose' (Lat. *osus*, signifying 'full of') added as a suffix to the genus of the causal fungus, with or without elision of the ultimate syllable of the generic name, in whole or in part, as may be determined by euphony."

[5] Through the kindness of Dr. W. A. ORTON of the U. S. Department of Agriculture, B. P. I., we learn that a Macrosporium disease of carnation was reported from Strassburg, Pa., in 1906, and one attributed to Alternaria from Connecticut by CLINTON in the same year.



1 2

FIG. 1. Single leaf showing diseased spot near base.—FIG. 2. Diseased plant showing removal of lower leaves by the gardener in his efforts to stop the progress of the disease.

what and becomes soft and disintegrated, resulting in the death of the more distal portions of the plant.

Variety of carnations affected.—A striking feature of this disease is its tendency to infect to a large degree one variety, the Mrs. Thomas W. Lawson, to the exemption of others. In all cases which have come to our notice, it has been this variety solely which was diseased; moreover, the only records that we find of the disease imply the same susceptibility.[6]

The causal fungus.—Throughout the diseased tissue of all spots occurs in great abundance a characteristic, dark, branching, septate mycelium (*fig. 3*). The surfaces of diseased spots in periods later than their earliest youth present an abundance of black cespitose hyphae arising from the stomata (*fig. 4*). Spores of the Alternaria type are found in abundance (*figs. 5, 6*), both *in situ* upon these hyphae, and strewn over the surface of the diseased spots between the hyphal bases. The character and arrangement of the hyphae are shown in *figs. 7* and *8*. This fungus was constantly associated with the disease, and no other fungus was found. The presumptive evidence was therefore very strong that this fungus was the cause of the disease. In view of the often saprophytic habit of Alternaria, conclusions on this point would not be valid without evidence from inoculations.

Inoculations.—The fungus was easily isolated by direct transfer of spores from the diseased spots to carnation-leaf agar plates.

On October 27 numerous inoculations were made upon two plants under bell jars, using small pieces of agar, bearing spores and mycelium. One of the plants was left uninjured and the inoculum was placed in the axils of the leaves; in the majority of these cases the inoculations resulted in infection. The other plant was injured by the prick of a needle at the point of inoculation. In these cases about two-thirds of the inoculations were successful. Inoculations with spore suspension were also made upon five branches each, of two other plants, and each was covered by a large test-tube plugged around the stem with cotton to preserve a humid atmosphere. As in the former cases, the inoculations on one plant were at injured points, and those on the other plant were at uninjured points. The results from these spore inoculations were the same as in the cases of inoculations with agar blocks. When these inoculations were made, others were made upon six other plants from the same spore suspensions, but the plants were not covered or injured in any way. Following these last inoculations no signs of the disease were seen. It seems from these experiments that the injured

[6] Orton, W. A., Yearbook, U. S. Dept. Agric. **1905**:611.

plant is readily susceptible to infection, as is also the uninjured plant if kept in humid condition, but that the uninjured plant in a relatively dry atmosphere is difficult or impossible to infect. In case of successful inoculation, the diseased spots were well developed at the end of a week. The removal

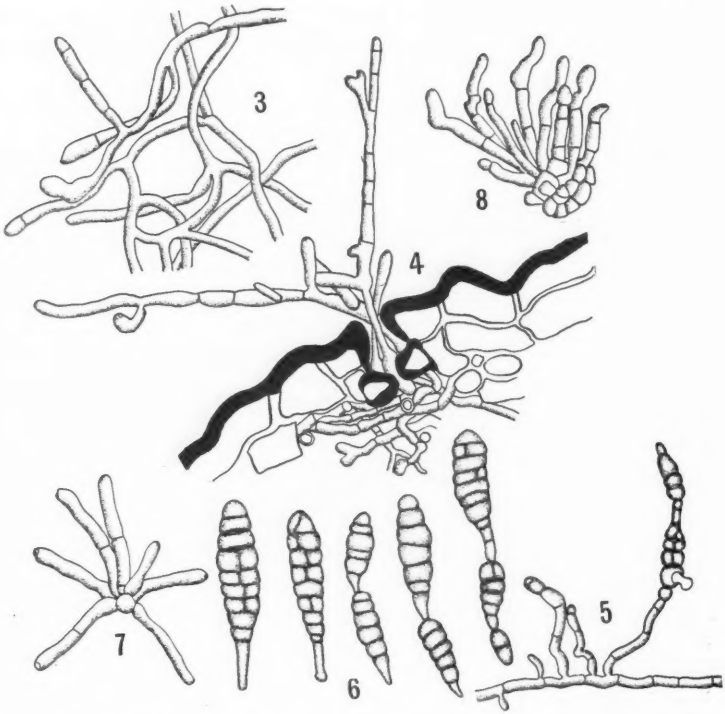


FIG. 3. Mycelium showing branching and septation.—FIG. 4. Showing mycelium below stoma and hyphae emerging through the stoma.—FIG. 5. Showing catenulate spores as borne upon hyphae.—FIG. 6. Spores showing shape, septation and catenulation.—FIG. 7. A young cluster of hyphae.—FIG. 8. An older cluster of hyphae.

of the protecting bell jar from plants already infected resulted usually in cessation of development of the spot. These facts agree well with the field observation that the most damaging infection occurs at the leaf axils, points well adapted to collect and hold water, thus providing conditions for optimum development of the fungus.

Culture characters.—The fungus was grown upon many different media. Its characters upon these media have been noted elsewhere.[7]

The most important culture characters may be summarized as follows:

Upon media poor in available carbohydrates the mycelium was nearly hyaline, and the hyphae and spores pale; upon media rich in carbohydrates the mycelium, hyphae, and spores were very dark. Upon the natural medium the spores were more regular and uniform in shape and were much larger than upon artificial media.

The species of fungus.—Of the Alternarias there seems to be only one, *A. longispora* McAlph., growing upon members of the pink family (Caryophyllaceae),[8] and the description of this does not agree with ours in size, shape, or septation of its spores.

Therefore, unless an attempt be made to identify this form with some one of the seven or more species of Macrosporium infecting the pink family, a procedure which would be unjustifiable without resort to cross-culture inoculations and extensive study in artificial media, this form had best be designated as a distinct species, for which we propose the following name and description:

Alternaria Dianthi, n. sp.—Hyphae cespitose from stomata, amphigenous, dark brown, 1–4-septate, ascending, 1–25 from each stoma; conidia 26–123×10–20 μ, catenulate, clavate, tapering, base obtuse, dark brown, slightly constricted at the septa, transverse septa 5–9, longitudinal septa 0–5; spot ashen white, definite, subcircular.

On artificial media poor in carbohydrates mycelium lighter in color, spores lighter, smaller, and with fewer septa.

HABITAT: living leaves and stems of *Dianthus Caryophyllus*, Raleigh, N. C.—F. L. STEVENS AND J. G. HALL, *N. C. Agricultural Experiment Station, West Raleigh.*

[7] STEVENS AND HALL, Variation of fungi due to environment, *ined.* Read before the Botanical Society of America at the Baltimore meeting, December, 1908.

[8] SACCARDO, Syll. Fung. **18**:638.

CURRENT LITERATURE

BOOK REVIEWS

Another mushroom book

Mushrooms, by reason of their beauty and edibility, are almost as attractive and popular as pretty wild flowers, and so we may expect to see popular guides to their collection and consumption multiply. The latest candidate for favor is a book by Mr. HARD, now superintendent of public instruction in Kirkwood, Mo., but for some years located in southern Ohio, where he became interested in collecting and studying these plants. Under the encouragement of KELLERMAN, ATKINSON, LLOYD, MORGAN, PECK, and other mycologists, he has evidently become an enthusiastic amateur. By his camera, supplemented occasionally by those of his friends, he has pictured a great number of representative specimens. Presenting these photographs, to the number of 500 and more, combined with descriptions, sometimes technical, but usually popular and more or less diffuse, he has prepared a ponderous volume.[1]

This volume, chiefly on account of its excellent half-tone illustrations, which include almost all of the common species, will be of good service to those who wish a book less expensive and voluminous than MCILVAINE'S, and at the same time comprehensive enough to enable them to identify the plants they pick up in fields and woods.

It is evident that the author has no adequate technical training in taxonomy or morphology; and in presenting such matters, neither his keen powers of observation nor his enthusiasm could prevent him from falling into errors both of form and fact. The typography of the book shows, also, that both author and publisher are unacquainted with scientific practice, while the proofreader and the author alike are responsible for many typographical errors. The etymology of the scientific names, by which the author hopes to show their significance to those unaccustomed to them, is often erroneous and occasionally ludicrous. The glossary does not define all the technical terms that are used, no less than four in a single description of eight or ten lines having been hit upon by mere chance. The list given of authorities for generic and specific names is far from complete, so that abbreviations used in the body of the text (which are not consistent) could not possibly be identified.

[1] HARD, M. E., The mushroom, edible and otherwise; its habitat and its time of growth, with photographic illustrations of nearly all the common species. A guide to the study of mushrooms, with special reference to the edible and poisonous varieties, with a view of opening up to the student of nature a wide field of interesting and useful knowledge. 4to. pp. x+609. *pls. 60. figs. 504.* Columbus, O.: The Ohio Library Co. 1908. $4.75.

All these things show the apprentice hand; but, though they mar the book, they do not so detract from its value that it may not be commended to the public for whom it is intended. It will indeed be a welcome addition to public, school, and college libraries, where there is always a demand for well-illustrated books of this kind, and it will probably do good service in awakening an interest in mushrooms. It certainly treats wisely the matter of testing the edibility of mushrooms and no one who follows Mr. HARD'S advice will come to harm. Thus it has a real field of usefulness. But it is not for the mycologist; and, unless "of its kind" is a saving clause, it is by no means what is claimed by the publishers in their circular—"By far the most complete work of its kind ever attempted in this country."—C. R. B.

Trees and woods

The fourth volume of the series on *Trees* by the late H. MARSHALL WARD has recently come from the Cambridge University Press.[2] With a fifth now in press the series will be concluded, for although the author had planned another, it was too inchoate to permit publication. The present volume has been issued under the editorial supervision of PERCY GROOM, who has left the manuscript practically unchanged, but has had the labor of selecting the illustrations, which are both numerous and appropriate.

As the three preceding volumes have treated respectively the buds and twigs, the leaves, and the inflorescences and flowers, this one presents the fruits. After a general discussion of the morphology of fruits (part I, 59 pp.), the second part (94 pp.) gives a key to trees and shrubs, based on characters derived from fruits, and accompanied by figures of most of the species. Like the other parts of the series, this one will doubtless be useful in Great Britain, though it cannot be particularly serviceable in this country. It embodies a good idea, however, which might be applied to the trees of this continent.

The eighth part of SCHNEIDER'S *Handbook of deciduous trees* (the third section of the second volume) has lately issued from the press.[3] Like its predecessors, frequently referred to in this journal, it presents, in the most compact form possible, descriptions of the species of angiospermous trees, native or planted out in central Europe, arranged in the sequence of a dichotomous key, and illustrated freely. It seems a most thorough and practical book, but somewhat disconcerting as to nomenclature. Who of our foresters will give us something as good, but perhaps a little less condensed?

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[2] WARD, H. M., Trees: a handbook of forest botany for the woodlands and the laboratory. Vol. IV, Fruits. Cambridge Biological Series. 12mo. pp. IV+161. *figs.* 147. Cambridge: University Press. 1908. New York: G. P. Putnam's Sons. $1.50.

[3] SCHNEIDER, C. K., Illustriertes Handbuch der Laubholzkunde. Charakteristik der in Mitteleuropa heimischen und im Freien angepflanzten angiospermen Gehölz-Arten und Formen mit Ausschluss der Bambuseen und Kakteen. Achte Lieferung (dritte Lieferung des zweiten Bandes). Imp. 8vo. pp. 241–366. *figs. 166–248.* Jena: Gustav Fischer. 1909. *M* 4.

The second part of JANSSONIUS' elaborate micrography of the woods of Java[4] has recently come from the press. In the notice of the first part of this work[5] we described the plan, which is here merely extended. The part includes in the concluding section of the first volume (Dicotyledones, Polypetalae, Thalamiflorae) descriptions of 67 species, in addition to the 108 of the first section. The second volume begins the Disciflorae and presents 54 species. The complete work will certainly be a monument of industry and will be serviceable for the microscopic identification of Javanese woods. That the game is worth the candle, we may be permitted to doubt.—C. R. B.

Microscopy of technical products

Now that so much attention is being paid to the purity of foods, drugs, and manufactured products of all kinds, it becomes of the greatest importance to have adequately trained men in municipal, state, and national offices, to whom can be submitted the many questions that are sure to arise as to the adulteration or sophistication of marketed articles. Unfortunately the number of competent persons is far short of the demand, and this state of affairs is sure to continue for some time. In this situation the only recourse is to have accurate handbooks in which may be found detailed descriptions of the characteristics, chemical and microscopic, of all the commoner substances which enter into commerce. Then one who has a reasonable familiarity with microscopic manipulation may be able to determine the more obvious cases of adulteration, and by experience may acquire real expertness.

To put into reach of American laboratories one of the most valuable of foreign works, Dr. A. L. WINTON, chief of the Chicago Food and Drug Laboratory of the Department of Agriculture, with the assistance of Dr. KATE G. BARBER, has translated an edition of HANAUSEK'S *Lehrbuch der technischen Mikroskopie*, which represents the last German edition extensively revised by the author.[6] The translators have also included a considerable amount of new material, and in particular they have incorporated into the key to economic woods—a most valuable feature of the book, permitting one to determine most of the species from a fragment of the wood—the American species of commercial importance. The illustrations have also been improved and augmented.

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[4] JANSSONIUS, H. H., Mikrographie des Holzes der auf Java vorkommenden Baumarten; im Auftrage des Kolonial-Ministeriums unter Leitung von Dr. J. W. MOLL bearbeitet im Anschluss an "Additamenta ad cognitionem florae arboreae javanicae auctoribus S. H. KOORDERS et TH. VALLETON." Zweite Lieferung. 8vo. Vol. I, pp. 369–568. Vol. II, pp. 1–160. *figs. 45–95*. Leiden: E. J. Brill. 1908. *M* 6.

[5] BOT. GAZETTE **43**:345. 1907.

[6] HANAUSEK, T. F., The microscopy of technical products. Revised by the author and translated by ANDREW L. WINTON, with the collaboration of KATE G. BARBER. Imp. 8vo. pp. xii+471. *figs. 276*. New York: John Wiley & Sons. 1907. $4.75.

The work opens with a section on the construction and use of the microscope, its accessories and reagents. The important types of technical products that are treated are the following: starches and inulin; vegetable fibers, including hairs, with a section on the examination of paper; animal fibers, mineral fibers, and textile fabrics; stems and roots, including woods (gymnospermous, dicotyledonous, and monocotyledonous), barks, and rhizomes, with some practical examples of the problems that are submitted for solution; leaves, under which only sumach leaves are treated; flowers, with insect powder alone treated; seeds and fruits, including a large range of oil cakes; and finally teeth, bone, horn, etc.

Of course in such a list there must be an end somewhere, for space is not unlimited; but one wonders at the basis of some choices. The line between drugs, foods, and technical products is not an easy one to draw; but if wheat and barley appear among the fruits, why not maize and rye? If sumach leaves, why not tea and tobacco? If insect powder, why not saffron? But it behooves us to be thankful for what there is, rather than to complain of what there is not. And what there is is sure to be thoroughly helpful.

The publishers' part has been well done. The illustrations are well printed, the text clear, and the binding substantial. The book is essential for public libraries and for governmental and university laboratories.—C. R. B.

Works of Léo Errera

We have already noticed in these pages the sumptuous republication of the work which, under the direction of LÉO ERRERA, issued from the botanical institute of the University of Brussels. In these volumes[7] his own original work takes a conspicuous place. But he did much other writing, popular, pedagogic, philosophic, literary, which is to be preserved by original publication or reprinting in a series of six volumes now being issued under the title *Recueil d'œuvres de Léo Errera*.[8] Of these three have appeared. Two deal with botanical subjects and one contains verse and prose on a variety of topics—addresses, thoughts, philosophic epigrams, etc. The botanical topics of the first volumes are: A letter on the vegetation about Nice; Agriculture and horticulture in Norway (largely a criticism of SCHÜBELER); Structure and modes of fecundation in flowers (200 pp.); Secondary heterostylic characters of primroses (a posthumous work completed by Miss J. WERY) (30 pp.); Progress of systematic botany; A neglected field of research (the efficacy of the defensive structures of plants); ENGELMANN'S bacterial method; Compass plants. In the second volume we find: Respiration of plants (one of a course of public lectures); De grâce, des noms latins (a plea for the avoidance of vernacular names); Scientific bases of agriculture (36 pp.);

[7] Recueil de l'Institut Botanique Léo Errera. BOT. GAZETTE **43**:215, 347. 1907; **45**:201. 1908.

[8] Recueil d'œuvres de LÉO ERRERA. 8vo. Vols. I, II, Botanique Général. pp. iv+341. Vol. VI, Melanges (vers et prose). pp. xiv+222. Bruxelles: H. Lamertin. 1908.

Descriptive text of physiological charts (90 pp., including small reproductions of the charts which he published in conjunction with LAURENT); Letter prefatory to DE WILDEMAN'S *Flore des Algues de Belgique;* An elementary lesson on Darwinism (106 pp.; an admirably clear and brief presentation, which appeared first in 1900 and is now printed as he had revised it for a third edition). This volume closes with three posthumous articles: Plants in contrast with other beings; What there is in a plant; The *épopée* of a ray of sunlight.

These volumes, as well as the more strictly scientific ones, will form a worthy memorial of this distinguished *savant*, whose writing is always luminous and inspiring. His bibliography, though voluminous (287 titles, as we learn from an interesting biography just published[9]), is remarkable, not alone for its extent, but for its value. To have all his work collected is a real boon.—C. R. B.

NOTES FOR STUDENTS

Papers on mucors.—Two valuable papers, largely taxonomic in character, have recently appeared on the mucors. In two ways they show an advance over other taxonomic work in this confused group. In the first place the center for fungus cultures maintained by the *Association Internationale des Botanistes* has been made use of, and the species investigated were compared as far as possible with named cultures from this and from other sources. Provided contamination of cultures in the source of supply is avoided, this center in Amsterdam affords a ready method of checking up determinations and should be of increasing value to mycologists. In the second place the differentiation of species according to their sexual character into homothallic and heterothallic forms is recognized as an item in the classification, and in heterothallic species the production of zygospores, when a given strain is grown in contact with the opposite strain of a known species, is used to establish its specific identity with the form tested.

HAGEM[10] announces his paper as a preliminary contribution to a study of soil mucors. By exposing Petri-dish cultures to the air and allowing the spores which fall on them to develop mycelial colonies, he finds with SAITO that spores of the mucors, both absolutely and relative to other molds, are unexpectedly of infrequent occurrence in the air. Only seven species were thus found. In investigating the mucor flora of the soil, samples from different kinds of soils were sown on various nutrient substrata, and the resulting growths isolated in pure cultures. Sixteen different species, confined to the genera Mucor, Rhizopus, Absidia, and Zygorhynchus, were found, of which six are described as new, viz., *Mucor strictus*, *M. sphaerosporus*, *M. griseo-cyanus*, *M. silvaticus*, *M. norvegicus*, and *Absidia glauca*. Four new forms are added to the list of heterothallic species. One of these, *Mucor hiemalis*, was especially investigated as regards the distribution in

[9] FREDERICQ, LEON, AND MASSART, JEAN, Notice sur LÉO ERRERA, membre de l'Academie. 12mo. pp. 153. Brussels: Hayez. 1908.

[10] HAGEM, OSCAR, Untersuchungen über norwegische Mucorineen, I. Vidensk.-Selsk. Skrifter. I. Math.-Nat. Kl. No. 7. pp. 50. 1908.

nature of the two sexual races. From a total of 52 separate isolations, 21 were of one sex, 5 of the other sex, and 26 (50 per cent.) failed to give any reaction with the test strains and were listed as neutral. Three of the strains that took part in zygospore formation were very weak in their sexual activity, and one of them under further culture entirely lost its power to take part in zygospore formation. The distribution of the sexual races in this species is thus shown to be in accord with the condition in Rhizopus, where out of 59 strains investigated by the reviewer, 19 were (+), 27 (−), and 13 neutral. The large percentages of neutral races thus established for these two species, together with the reviewer's own experience with neutral races in other heterothallic species, renders it probable that sexual neutrality is a widespread phenomenon among the mucors. There is little at present known to indicate its cause or significance.

LENDNER,[11] in his studies of the Mucorineae of Switzerland, has not confined himself to mere local species, and though he has not attempted to present an exhaustive treatment of the whole group, he has given us a more or less critical arrangement of the genera Mucor, Rhizopus, and Absidia. In these three genera keys for the determination of species are given, and each form is described, either from the original description or from A. FISCHER, with additional notes on such species as he had himself cultivated. In classifying the genus Mucor, FISCHER's division into the unbranched, racemosely branched, and cymosely branched groups is followed. Fifty-one species are recognized, of which seven, *M. lausannensis*, *M. genevensis*, *M. pirelloides*, *M. lamprosporus*, *M. Jansseni*, *M. spinescens*, *M. dimorphosporus*, are described as new species, and one, *M. adventitius* var. *aurantiaca*, as a new variety. BAINIER's genera, Parasitella and Glomerula, are reduced to the genus Mucor, as also VUILLEMIN's Zygorhynchus. The genus Mucor is the Crataegus among fungi and will probably always remain a taxonomic playground for mycologists. One might imagine that early systematists used the genus as a group to practice on, and their one- or two-line descriptions are frequently hardly sufficient to tell us whether the form described is a mucor or a myxomycete. Such supposedly common forms as *Mucor Mucedo* and *M. racemosus* among others, it is impossible to determine with any degree of accuracy, and therefore these designations can be considered hardly more than group names. We cannot but have considerable charity toward one who feels inclined, in consequence, to disregard the stock names, but when each mycologist who works on the genus gets out a list with names of his own, the result is confusing to a degree. Moreover, species shown to be distinct by the reaction between their sexual strains are frequently so closely related and vary so widely under different conditions of cultivation that the usual description is insufficient to distinguish them. LENDNER has done a service in bringing together the descriptions of species since FISCHER's publication. We are grateful that he has not found it necessary to make new species out of more than 15 per cent. of the 51 forms

[11] LENDNER, ALF., Les Mucorinées de la Suisse. Matériaux pour la flore cryptogamique Suisse. Vol. III, Fasc. I. pp. 180. 1908.

listed. Seven circinellas are described, of which *C. minor* and *C. aspera* are given as new. In the genus Rhizopus, of which 22 species are recognized, physiological characters, such as ability or inability to grow on potato above 39° C. and power to ferment different carbohydrates, are used in addition to the usual distinguishing morphological characters. Material received from the Amsterdam center under the name of *Mucor norvegicus* is identified as *R. nodosus*. Seventeen species are recognized in the genus Absidia, of which *A. spinosa*, a homothallic and heterogamic species, is described as new. In addition to the forms from the genera mentioned, *Cunninghamella elegans* is described as new.

In addition to the systematic part of 113 pages, an introduction of 47 pages is devoted to methods of isolation and cultivation, and to a discussion of the sexual reproduction in the group, together with the results of a cytological investigation of the formation of zygospores. It might be expected that forms in which the sexual differentiation had extended to the separation of distinct male and female races would show a differentiation in the uniting gametes. In no heterothallic form, however, has there been shown to be any constant difference in the size of the gametes, such as occurs in a few of the homothallic species, where, since the zygospores are produced between neighboring filaments of the same plant, a less specialized sexual condition might be supposed to exist. In *Absidia Orchidis*, LENDNER finds that the circinate outgrowths, which typically arise from both suspensors, are at times produced from but one, which has been cut off from the large progamete that he considers female. This he claims an indication of sexual differentiation, as also the frequent inequality in the gametes of Rhizopus. From these facts he concludes that the (+) and (−) races are potentially homothallic, but with the opposite sex more or less completely suppressed. The suggestion that the sexual races may be potential hermaphrodites is in line with our knowledge of higher forms, but to formulate this as a conclusion and to claim that the smaller and larger gametes formed by a single sexual race are male and female respectively, as LENDNER would imply, is certainly going beyond the facts in hand. The reviewer has shown that in Rhizopus the larger gamete is derived sometimes from the (+) and sometimes from the (−) plant, and that similarly in the heterothallic species of Phycomyces the outgrowths (which LENDNER, p. 38, wrongly says arise from the zygospore itself) are confined sometimes to the (+) and sometimes to the (−) suspensor. The inconstant difference in size of gametes and behavior of outgrowths from the suspensors in *Absidia Orchidis* is probably merely nutritive in character and of no sexual significance. In *A. Orchidis* also, is figured what appears to be an arrested stage in the formation of a zygospore between two outgrowths from the same suspensor, and therefore belonging to the same sexual race. If this is used as an argument for the contention just mentioned, the fact should be established beyond doubt. Even if the author were not mistaken as to the terminations of the filaments apparently in conjugation, which would be difficult to follow in a tangle of other filaments, these two arrested gametes might be thought to have arisen adjacent to each other at the stimulus of contact of a third branch, which came from the opposite sex but had remained in only temporary contact with them.

No two investigators are as yet in accord as to the cytology of the zygospores of the mucors. GRUBER, who apparently has done the most careful work on the zygospores of Sporodinia, was unable to find a fusion of nuclei at any stage in their formation or maturation, and several of the most experienced of American and European cytologists of the fungi have personally told the reviewer that they also have investigated the zygospores of this same species, but with no better results. In 1906, DANGEARD, working with *Mucor fragilis*, described the uniting cells as gametangia and saw a fusion of nuclei in pairs soon after the union of the two sexual cells. The condition in Sporodinia was more difficult to follow, but DANGEARD believed he was able to find the same condition in the zygospores of this species. LENDNER, in the work before us, criticizes the conclusions of DANGEARD, claiming that the figures which DANGEARD interprets as stages in fusion are in fact stages in division, since they occur at the same time in the two suspensors as well. What DANGEARD considers as degenerating supernumerary nuclei toward the periphery of the zygospore, LENDNER never finds in degeneration, and he believes them to be in this position to preside over the formation of the membrane. The real sexual fusion, according to LENDNER, is between two large nuclei which approach the center of the zygospore. The two densely staining bodies in the fusing nuclei, which are homologized with chromosomes, give at first four bodies in the fusion nucleus, that eventually are reduced to two and finally unite into a single mass. Neither DANGEARD nor LENDNER has studied the germination of the zygospores.

Since KLEBS showed that external factors are responsible for the form of reproduction in Sporodinia and many other fungi, the influence of external conditions upon the growth and reproduction of individual species has become a favorite subject of investigation. As the reviewer has shown, external conditions are more influential in determining the form of fructification in the two homothallic species of Sporodinia and Dicranophora, found growing on fleshy fungi, than in the homothallic species, *Zygorhynchus Moelleri*, recently investigated by WISNIEWSKI,[12] a pupil of RACIBORSKI. It seems to be generally true in regard to the influence of external conditions, that the limits within which zygospore formation is possible are narrower than those within which sporangial formation occurs. WISNIEWSKI finds that, although under ordinary conditions sporangia are formed together with zygospores on the same mycelium, extreme conditions may suppress the production of zygospores, while sporangia are still formed. On pure agar below 5° C. and on the same substratum in direct sunlight, only sporangia will be produced. (It may well be the heat rather than the light effect of the direct rays of the sun that is here influential.) Under all other conditions examined, both zygospores and sporangia were produced together, if at all. The decrease in relative abundance of zygospores is associated with a checking of the rapidity of growth of the mycelium. The transpiration is shown to have no effect upon the

[12] WISNIEWSKI, P., Einfluss der äusseren Bedingungen auf die Fruchtform bei *Zygorhynchus Moelleri*. Bull. Acad. Sci. Cracovie Cl. Sci. Math. et Nat. **1908**: 656–682.

rapidity of growth, and it is assumed that for this reason increase or decrease of moisture in the surrounding air does not affect the proportion between zygospores and sporangia. Upon certain substrata, zygospores are more abundant at the junction of adjacent mycelial colonies, forming dark lines. The conditions governing their production have not been investigated.

For several years DAUPHIN has been interested in the genus Mortierella. In a recent paper on the genus[13] he gives in 28 pages a systematic arrangement of the species as an introduction to a special study of *M. polycephala*. The original descriptions and figures are given for each of the 29 species and varieties discussed. Two new species, *M. canina* and *M. raphani*, and one new variety, *M. van Tieghemi* var. *cannabis*, are described. The genus is divided into four group species (*grandes espèces*) with subspecies (*petites espèces*) and varieties under them. A well-arranged key would have added to the value of this part of the work. *M. polycephala* is the only one of the Mortierellas which has been investigated in regard to the influence of external conditions upon the production of zygospores. In this species, which forms the subject of the physiological part of the paper, DAUPHIN has succeeded in finding the zygospores, and since he obtains them from sowings of single spores, he classifies the species as homothallic. This being the case, it seems strange that they have not been found in this form by other investigators, since as yet no neutral strains have been found for homothallic species. The optimum temperature for germination of the spores is placed at 27° C., and the optimum for formation of sporangia and zygospores, between 15 and 20° C. Germination and growth are checked by darkness, but the fructifications are not altered. Light increases the rapidity of development, but if too intense causes the fructification to be confined to stylospores. The violet and ultra-violet rays of the spectrum seem necessary for the germination of the spores. X-rays and the influence of radium are inhibitive to germination and growth, the radium in addition causing the production of cysts in the hyphae. Moisture in the surrounding air is shown to be necessary for the germination and growth of the fungus. Perfect development will take place in an atmosphere completely free from oxygen. A decrease, however, of atmospheric pressure below 150^{mm} causes the mycelium to remain sterile. An increase of pressure above atmospheric checks the growth of the mycelium without preventing the normal fructifications. *M. polycephala*, like other mucors, develops poorly in liquid media. The monosaccharids, especially dextrose and levulose, were found most favorable of the carbohydrates for formation of zygospores and sporangia. The influence of different concentrations of the nutrient was little investigated, but stylospores and sporangia were produced without zygospores when the amount of dextrose was increased from 20 per cent. to 60 per cent. The purpose of this part of the paper seems to have been to find out the influence of a large number of more or less isolated external conditions upon the form of fructification, rather than to work out thoroughly the influence of a few closely related factors.

[13] DAUPHIN, JEAN, Contribution à l'étude des Mortierellées. Ann. Sci. Nat. Bot. IX. 8:1–112. 1908.

It is hardly necessary in a botanical publication to comment on a recent note in *Science*, entitled *Mucor cultures*.[14] The author states that "in the study of the Mucoraceae for several years, some interesting facts concerning the development or rather non-development of zygospores were observed." In a thousand cultures of *Rhizopus nigricans*, made from material collected by the author or sent him by friends, as well as in five hundred specimens found growing spontaneously in different places, and in about five hundred other unrecorded observations of the fungus (a total of two thousand observations), no zygospores were found. Inoculations were made on a number of different media, including HAMAKER's corn-bread-muffin combination, and the growth of the cultures was tested in closed jars in H, N, and CO_2. No zygospores were obtained, and the conclusion is reached that "the absence of oxygen is not a necessary condition for the growth of zygospores." It is a pity that one who has the time to make observations on 2000 cultures should not have taken the trouble to read the recent literature on the subject, to learn in what part of a culture zygospores are produced and under what conditions their formation is possible, and so be in a position to make a contribution of some value. Additional information in regard to the relative distribution in nature of the two sexual strains of this most common of molds might have been the fruit of so extended a series of observations.

Appended is a list of species, the thallic condition of which has been determined, arranged according to the type of their sexual reproduction. Following each species is given in parentheses the name of the author who has investigated the zygospores and determined the sexual condition of the species in question.

Homothallic

1. Sporodinia grandis (BLAKESLEE)
2. Spinellus fusiger (BLAKESLEE)
3, 4. Mucors I and II (BLAKESLEE)
5. Mortierella polycephala (DAUPHIN)

Heterogamic

6. Dicranophora (BLAKESLEE)
7. Zygorhynchus Moelleri (BLAKESLEE)
8. Zygorhynchus heterogamus (BLAKESLEE)
9. Abisdia spinosa (LENDNER)

Heterothallic

1. Mucor Mucedo (BLAKESLEE)
2. M. hiemalis (HAGEM)
3. M. silvaticus (HAGEM)

4–9. Mucors III–VIII (BLAKESLEE)[15]

10. Absidia Orchidis (HAGEM)
11. Absidia glauca (HAGEM)
12. Absidia caerulea (BLAKESLEE)
13. Absidia repens (BLAKESLEE)
14. Rhizopus nigricans (BLAKESLEE)
15. Phycomyces nitens (BLAKESLEE)
16. Circinella umbellata (BLAKESLEE)
17. Cunninghamella echinulata (BLAKESLEE)
18. Choanephora cucurbitarum (BLAKESLEE)
19. Helicostylum piriforme (BLAKESLEE)
20. Syncephalastrum (BLAKESLEE)
21. MUCOR N, n. gen. (BLAKESLEE)

—A. F. BLAKESLEE, *Storrs, Conn.*

[14] SUMSTINE, DAVID R. Science N. S. **29**: 267. Feb. 12, 1909.

[15] Perhaps some of Mucors III–VIII are identical with species already in the list.

Mesozoic Equisetales.—One great desideratum in discussions as to the origin of existing plants is an increase in our knowledge of those of the Mesozoic. At the present time the Paleozoic flora is much better understood than that of the intervening period, which gave rise to the characteristic groups of our existing flora. A contribution by HALLE[16] throws a good deal of light on the organization of the equisetum-like forms of the earlier Mesozoic (Upper Triassic and Lower Jurassic). The author describes the vegetative stems and cones of several Equisetales. He establishes a new genus, *Neocalamites*, which has the general habit of the Calamites, including the leaf whorl made up of ununited leaves, with the herbaceous texture of the existing equisetums. It further resembles Calamites in the fact that only every second internodal strand (or fewer) gives off a leaf trace in the region of the node, and in the fact that the internodal bundles are frequently continuous at the nodes, in contrast to the alternating condition found in Equisetum. In Equisetites the leaves are in united sheaths as in the living genus, but in some of the species described by the author the same continuous bundles, and leaf traces fewer than the internodal strands, as are found in the Paleozoic Equisetales, are described. In the smaller branches, however, the leaf strands correspond to the number of internodal strands, thus foreshadowing the condition found in the living Equisetum. Perhaps the most interesting feature of this important addition to our knowledge is the description of the cones, cone-scales, and spores of Equisetites. The two former do not differ strikingly from those of the living genus, but the spores, interestingly enough, show the absence of elaters and the presence of triradiate sculpture described for the megaspores and microspores of the Calamites. The cones are isosporous. This article connects in a very satisfactory way the organization of the Paleozoic Equisetales with that of those still living, and illustrates the important bearing of paleontological facts on any stable scheme of evolution.—E. C. JEFFREY.

Membrane of diatoms.—MANGIN presents[17] an account of some extended observations on the diatoms, especially those of the plankton. His most important observations relate to the membrane. This he finds to be composed of a substance identical with pectic compounds, combined more or less intimately with silica; the siliceous skeleton thus formed is impregnated and invested with a gelatinous membrane which often hides, at least in plankton species, the characteristic ornaments. He controverts the ideas of SCHÜTT as to the growth of the membrane (through agency of an extracellular plasma), which he discusses at some length; and after describing improved methods of staining the membrane (by ruthenium red, and by an *old* solution of hematoxylin with ammonium- or ruthenium-alum, which may be aged artificially), he gives some detailed examples in the study of certain species.—C. R. B.

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[16] HALLE, T. G., Zur Kenntniss des mesozoischen Equisetales Schwedens. Kung. Svensk. Vetenskapsakad. Handl. **43**: No. 1. 1908.

[17] MANGIN, L., Observations sur les Diatomées. Ann. Sci. Nat. Bot. IX. **8**: 117–219. *figs. 14.* 1908.